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# Phytolith analysis as a means of cellulosic fibre identification: Silica bodies and calcium oxalate crystals in *Agave* genera (*cantala*, **maguey** and *sisalana*, **sisal**), *Boehmeria nivea* (L.) Gaud. (**ramie**), *Cannabis sativa* L. (**hemp**), *Corchorus capsularis* L. (**jute**), and *Musa textilis* Née (**abaca,** or **Manila hemp**)

by

Yolanda Margaret Deirdre Olivotto ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

in

Clothing and Textiles

Department of Human Ecology

Edmonton, Alberta

Spring, 1996

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Department of Human Ecology
115 Home Economics Building
University of Alberta
Edmonton, Alberta
T6G 2M8
January 19, 1996

Yolanda Olivotto
1790 Broadmead Avenue
Victoria, B.C. V8P 2W1

Dear Yolanda:

Of course you may have permission to use the image of the protocol from my thesis to use in yours. If you need it stated more formally for your files let me know.

Sorry it took so long for me to get back to you, but, as you can see, I'm here at the U. of A for 4 months replacing Heather. It's extremely cold and I have a lab to teach this afternoon for which I feel insufficiently prepared. Oh well, that can't be helped. It's baptism by fire on very short notice.

I'm glad things are progressing well for you.

Joan Marshall

Joan Marshall

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Nancy Kerr

Dr. Nancy Kerr

David D. Cass

Dr. David D. Cass

Betty Crown

Dr. Elizabeth Crown

Date Approved: 4 April, 1996

# ABSTRACT

The study evaluates phytolith analysis as a means of cellulosic fibre identification for use by conservators, and contributes to phytolith reference collections. Seven methods of phytolith distillation are evaluated and the phytolith content of a set of cellulosic materials is presented. Study materials include *Agave* (*cantala*, **maguey** and *sisalana*, **sisal**), *Boehmeria nivea* (L.) Gaud. (**ramie**), *Cannabis sativa* L. (**hemp**), *Corchorus capsularis* L. (**jute**), and *Musa textilis* Née (**abaca**, or **Manila hemp**). Study methods include mechanical sectioning, chemical digestion, thermal ashing, and oxygen plasma ashing. Each study material contained silica bodies and/or calcium oxalate crystals that were either a) aligned with the fibre and fibro-vascular bundles and connected to the bundles by way of their containment cells (lithocysts), or b) present as individual phytoliths in more processed materials. The association of phytoliths with plant fibres indicates that phytolith analysis could evolve to be an appropriate identification tool for use by conservators.

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Thank you to fellow Human Ecology grad students, "Brainstormers" Joëll, Laurie, Effy, Shawna, Jenn, Mari, Kaye, and Anne, as well as Terri and José, for assisting in making my work more enjoyable. And, thank you to my friends from the textile conservation lab, Gaby, Heather, and Margaret, for adding laughter to my days. I also thank my new friends in Edmonton, Greg Fulton, the Batemans, and the Cottrells, for making me feel like family, and my new *Great* cousin Blake McDougall and his wife, Norma, for their kindness, generosity, and emotional support.

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**c)** *Cannabis sativa* L. (**hemp**).

# CHAPTER 1: INTRODUCTION

## Background To The Research

The current study complements on-going research at the University of Alberta, Department of Human Ecology, which is directed towards elucidating a technique for the identification of highly degraded cellulosic textiles. The work of previous students revealed that highly degraded cellulosic fibres have lost their characteristic morphologies and do not react to chemicals in a predictable way (Marshall, 1992). As well, a student identified the possibility that phytolith analysis, or the study of mineralized inclusions (including crystals of calcium oxalate and silica) from plant tissues may provide a key to identifying highly degraded cellulosic materials (N. Kerr, personal communication, February 10, 1994). A review of the literature revealed that the systematics of phytolith analysis involves a) the development of reference collections, b) the distillation of residual phytoliths from a soil or other matrix, c) the classification of the distilled phytoliths, and d) the comparative analysis of the distilled phytoliths to the reference collections. The development of the reference collections involves processing plant specimens to reveal their content of phytoliths, and classifying the phytolith yield. The reference collections are then the keys used in the identification of plant populations based on phytolith assemblages that are from soil or other matrices. The purpose of the current research was to determine if phytolith analysis is a viable procedure for use by textile conservators in the identification of highly degraded cellulosic materials. As a model, the research employed phytolith systematics. The materials that were studied included *Agave* genera (**maguey** and **sisal**), *Boehmeria nivea* (L.) Gaud. (**ramie**), *Cannabis sativa* L. (**hemp**), *Corchorus capsularis* L. (**jute**), and *Musa textilis* Née (**abaca**, or **Manila hemp**).

The conservation and interpretation of artifacts made from cellulosic materials commences with description, including materials identification. Specificity of identification is at times necessary, however, the identification of highly degraded cellulosic materials is sometimes not possible using conventional methods. As a result, conservators require a method of identification that involves the analysis of a characteristic that persists through degradation. Phytolith analysis, or the study of residual mineralized inclusions from plant tissues, is a method used by archaeologists and paleoecologists to characterize previously extant plant populations. Furthermore, crystals of calcium oxalate and silica have been studied by botanists for various reasons, including taxonomy. Phytolith analysis is a possible identification tool for use by conservators as a result of the apparent fact that phytoliths are a constant in live as well as highly degraded cellulosic materials. As a result, conservation scientists may choose to consider incorporating phytolith analysis into the hierarchy of currently established cellulosic material identification protocols for use when specificity of identification is necessary.

## Barriers To Identification: The Research Justification

The literature that provided the point of departure for the research included Marshall's (1992) masters thesis, The identification of flax, hemp, jute and ramie in textile artifacts, and one source that she cited, Catling and Grayson's (1982) book, Identification of vegetable fibres. Both the thesis and the book provided the impetus for further research. Marshall concluded her study with the following two recommendations for further research: a) the testing of her protocol with other known soft and hard bast fibres, and with dyed fibres in order to expand the usefulness of the protocol and b), to study the crystals found in the plant species with which she worked, and to work with the ashing technique outlined in Catling and Grayson, in order to identify characteristics that persist through degradation, to thereby complement the protocol. The current research focused on the second recommendation.

The need to determine a characteristic that persists through degradation is evident when one reviews the difficulty that Marshall (1992) had in identifying some of the highly degraded unknown fibres with which she worked. In her work Marshall examined the morphological and chemical reactivity of four fibres of European economics; "flax, hemp, jute and ramie, were chosen for study...because, historically, their cultivation and use go back several thousand years. They are also all still in wide use today" (p. 4). Marshall developed a protocol which incorporated light microscope examination (for fibre morphology and diameters), drying twist characterization, and a chemical reactivity hierarchy (see Appendix A). She used the protocol to a great degree of success in the identification of some of the unknown samples with which she worked. However, when the fibres being tested were highly degraded, some of the tests could not be applied or gave inconclusive results; for example, the twist test could not be done on brittle fibres coated with debris. Additionally, the presence of soil debris sometimes masked the characteristic colours of certain diagnostic tests (pp. 118 - 128).

Catling and Grayson (1982) suggested an ashing method for distilling the crystals of calcium oxalate and silica from fibre samples, and they provided transverse, or cross, and radial longitudinal section drawings of a selection of plants, including the four studied by Marshall (1992). In a summary of their findings Catling and Grayson present the similarities and differences in the crystalline inclusions to be expected in ten different plant species (see Table 1). According to their summary, the four species of bast fibres that Marshall worked with should be distinguishable based on a study of their crystalline inclusions that reportedly persist through the ashing process.

Catling and Grayson (1982) reported that *Corchorus capsularis* L. (**jute**) exhibits cubic (rhomboid) crystals in chains sometimes mixed with occasional cluster crystals, or druse crystals (Esau, 1965); *Cannabis sativa* L. (**hemp**) has druse crystals in short chains, often three or four together, single druse crystals and the very occasional prismatic, cubic or rhombic, crystal; *Boehmeria nivea* (L.) Gaud. (**ramie**), has druse crystals in chains and single druse crystals; and *Linum usitatissimum* L. (**flax**), exhibits no crystalline inclusions. Each of Catling and Grayson's descriptions is slightly different, though in subtle ways. The authors reported that, predominantly, *Corchorus capsularis* has chains of rhomboid crystals, *Cannabis sativa* has short chains of druse crystals with occasional rhomboids, *Boehmeria nivea* has longer chains of druse crystals, and *Linum usitatissimum* has no reported crystals.

Within the four species there was no hydrated silicon dioxide, silica, identified by Catling and Grayson. However, they did report the presence of silica in *Musa textilis* Née

**Table 1.** Crystals and silica in ashed specimens of fibre.

| *Species* | *Crystals and silica in ash* |
|---|---|
| *Corchorus capsularis*<br>*Corchorus olitorius* | Cubic crystals in chains sometimes mixed with occasional cluster crystals.<br>Single cluster crystals. |
| *Hibiscus cannabinus*<br>*Hibiscus sabdariffa* | Cluster crystals in chains; Single cluster crystals. Only *very* occasional cubic or rhombic crystals in some specimens. |
| *Cannabis sativa* | Cluster crystals in very short chains, often three or four together. Single cluster crystals. *Very* occasional cubic or rhombic crystals in some specimens. |
| *Boehmeria nivea* | Cluster crystals in chains. Single cluster crystals. |
| *Crotalaria juncea* | Rhombic crystals, sometimes twinned.<br>Large amounts of fragmentary birefringent material in every specimen examined. |
| *Linum usitatissimum* | No crystals reported or seen. |
| *Agave sisalana* | Large acicular crystals, sometimes with organic matter annealed to the surface. |
| *Musa textilis* | Silica bodies.<br>*Very* occasional small acicular crystals in some specimens. |

**Note.** From Identification of vegetable fibres (p. 66) by D. Catling and J. Grayson, 1982, New York: Chapman and Hall in association with Methuen, Inc. Copyright 1982 by D. M. Catling and J. E. Grayson. Reprinted by permission.

**Note.** The common names of the plants referred to in Table 1 include **jute** (*Corchorus capsularis* and *Corchorus olitorius*); **kenaf** (*Hibiscus cannabinus*); **roselle** (*Hibiscus sabdariffa*); **hemp** (*Cannabis sativa*); **ramie** (*Boehmeria nivea*); **Sunn hemp** (*Crotalaria juncea*); **linen**, or **flax** (*Linum usitatissimum*); **sisal** (*Agave sisalana*); and **abaca**, or **Manila hemp** (*Musa textilis*).

(**abaca**, or **Manila hemp**), as did Schaffer (1981a), which indicates that they were looking for silica as well as calcium oxalate. Bozarth (1993) reported that *Boehmeria* has a type of "stalked verrucate" (p. 96) silicon dioxide inclusion, or phytolith as he named them. Another researcher, Nakamura, (1969), illustrated the epidermal trichomes, hairs, of *Boehmeria*, and suggested that they are silicified. Bozarth and Nakamura were likely writing about the same phenomenon. *Cannabis* is also known to have silicon dioxide, as well as calcium carbonate trichomes (Dayanandan & Kaufman, 1976). The effervescence that is noticed when dilute HCl is added to a slide containing leaf epidermis from *Cannabis* is a sign of the presence of calcium carbonate in epidermal trichomes (Nakamura). No other reports of silicon dioxide in *Corchorus*, *Cannabis*, *Linum* or *Boehmeria* were found.

The highly degraded nature of a textile fibre can be a barrier to identification using conventional methods. Even though the classifying of a set of characteristics that persist during degradation may be possible, there may still be other barriers that inhibit identification. Some other factors that could be barriers to identification are related to available resources. A skilled examiner, good reference collections, the necessary equipment, and economics all come into play with any project, especially with public conservation projects. Lastly, and most importantly, a barrier to specific identification can be the artifact itself. There are various reasons that an artifact may not be subject to examination. One of those reasons is that it may not be able to withstand the removal of a single fibre for identification; the fibres that are removed for study will be destroyed. It is always necessary to seek to achieve a balance: when it is legitimate and possible to work with an artifact, and when some other solution is appropriate.

## Research Purpose

At present, the protocol for the identification of cellulosic materials does not always lead to a conclusive identification. As a result, an underlying purpose of the research was to develop further the lab protocol for cellulosic materials to assist in the identification of highly degraded cellulosic materials. To that end, the main purpose of the research was to evaluate critically phytolith analysis as a potential tool in the identification of highly degraded cellulosic materials. The research proceeded in three phases: the first phase was conducted to evaluate the methods of phytolith distillation, the second phase was conducted to determine if phytolith information can be found in processed materials and manufactured textile products, and the third phase was conducted to determine if phytolith analysis is appropriate for use in identifying textiles materials other than the one studied in phase one and two.

### Phase I

The purpose of the first phase of the research was to evaluate seven methods of phytolith distillation based on phytolith yield, cost, time involvement, availability of equipment and supplies, and ease of equipment operation. Each of the seven methods that were evaluated is being used to distill phytoliths from plant specimens. Each method has its proponents, and yet most of the methods have also met with criticism. *Musa textilis* (**abaca**, or **Manila hemp**) was chosen for phase one because the discussions in the literature suggest that it contains a representative assortment of phytoliths, and because it is an important source of textile fibres. The results of the first phase of the research determined the methods that were used in the second phase of the research.

### Phase II

The purpose of the second phase of the research was to further evaluate the methods of phytolith distillation based on phytolith yield from various *Musa textilis* materials, representing various stages of production (leaf sheath and three grades of fibres, as well as paper and pulp). The possibility existed that in phase one of the research there would be no significant difference between two or more methods. The differences in the methods would become apparent once they are tested on different materials. One method may have proven to be more appropriate for certain kinds of materials, while another may have been appropriate for other kinds of materials. Additionally, the processing of plant materials so that they may be used in textiles may result in a loss of phytolith data (or a distortion of the data[1]). The phytolith yields from textiles may or may not correlate to the phytolith yields from harvested plant materials (for instance, in distinctivness or quantity).

### Phase III

The purpose of the third phase of the research was to determine if phytolith analysis was viable for a varied selection of plant materials used in textiles. In this last phase of the research, *Agave* genera (*cantala*, or **maguey**; and *fourcroydes*, or **sisal**), *Boehmeria nivea* (L.) Gaud. (**ramie**), *Cannabis sativa* L. (**hemp**), and *Corchorus capsularis* L. (**jute**) textile materials were studied for their content of phytoliths. In the case of *Corchorus* and *Boehmeria*, existing phytolith reference sources were used as keys to identification of phytoliths. In the case of *Agave* and *Cannabis*, plant specimens as well as existing phytolith reference sources were used as keys to identification of phytoliths.

## Specific Objectives

The specific objectives of the three phases of the research were:

**in Phase I,**

a) to measure and describe the phytolith yield from *Musa textilis* Née (**abaca**, or **Manila hemp**) using seven methods of phytolith distillation;

b) to calculate costs, measure time frame, and establish relative availability of equipment and supplies for seven different methods of phytolith distillation;

c) to compare and evaluate seven different methods of phytolith distillation;

**in Phase II,**

d) to measure, describe, and compare the phytolith yield from six *Musa*

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[1] The phytolith yield of processed materials may very occasionally increase as a result of processing. For example, Musa textilis is processed in Japan in a way that may introduce phytolith data that was not originally in the fibres (Norton, 1990, p. 121). At first this may appear to be a dilemma. However, when textiles are contextualized the additional phytolith data may, in fact, contribute to the positive identification of the material.

*textilis* Née (**abaca**, or **Manila hemp**) materials using one or more phytolith distillation methods, as determined by the results of phase one;

e) to further compare and evaluate methods of phytolith distillation;

**in Phase III,**

f) to measure and describe the phytolith yield from artifacts of known material origin;

g) to compare phytolith yields from harvested and processed plant materials;

h) to determine if there is a consistency in phytolith yields from plants, and from textiles made from the plants; and

i) to determine if phytolith analysis could be a viable aid in the identification of highly degraded cellulosic materials.

### Delimitations And Limitations Of The Study

The study did not plan to make a conclusive statement about the usefulness of phytolith analysis beyond its potential use in identifying textile fibres, such as the five being considered in the study. The study did not attempt to employ any methods of identification that are not readily accessible (such as DNA sampling), or are not appropriate to highly degraded cellulosic materials (such as fibre morphology or chemical reactivity studies). A final delimitation was that the study did not incorporate a phase that involved examining unknown highly degraded cellulosic materials.

The study was limited by the accessibility of materials from various stages of the harvest/product manufacture processes. In retrospect, the study would have benefited from having a variety of materials from each stage of harvest and manufacture from *each* of the species that were studied. In particular, the study would have benefited from having plant specimens of *Boehmeria nivea* (L.) Gaud. (**ramie**) and *Corchorus capsularis* L. (**jute**) to provide first-hand phytolith references. The study was also limited as a result of a lack of quantification methods.

## Definition Of Terms

*Ashing* (see *Cold Plasma Ashing and Thermal Ashing*).

*Bast Fibres, Hard*, are the highly lignified sclerenchyma cells, or fibres, from monocotyledon leaves, such as Musa textilis (**abaca**, or **Manila hemp**), Agave cantala (**maguay**), or Agave sisalana (**sisal**) (Esau, 1965).

*Bast Fibres, Soft*, are the nearly completely non-lignified sclerenchyma cells, or fibres, from dicotyledon stems, such as Boehmeria nivea (**ramie**), Cannabis sativa (**hemp**), Corchorus capsularis (**jute**), or Linum usitatissimum (**linen**, or **flax**) (Esau, 1965).

*Calcium Oxalate*, $CaC_2O_4 \cdot nH_2O$ in general formula, is an insoluble salt of calcium ions and oxalic acid anions. The ultimate production of calcium oxalate from its constituents appears to be "the result of a maintenance of ionic balance by the plant" (Kausch, 1983, p. 20), and may be reversible. Ishii and Takiyama report that it "is developed to protect protoplasm from toxic action of oxalic acid which is an end product of metabolism" (1989, p. 423).

*Cellulosic* is an adjective used to describe materials composed of cellulose $(C_6H_{10}O_5)_n$, a long chain organic polymer composed of β-d-glucose units. Two glucose units combine to make a cellobiose unit, which is the repeat unit of the cellulose polymer (Florian, 1990).

*Chemical Digestion*, involves the dissolving of materials using chemicals, sometimes initiated with a chemical catalyst, and sometimes initiated with thermal energy.

*Cold Plasma Ashing* involves ashing of organic materials using charged oxygen at a temperature not exceeding 100°C (Hozumi, Hutoh, & Umemoto, 1972).

*EDXA* (Energy Dispersive X-Ray Analysis) involves the use of reflected x-rays to determine elemental species present in a sample (G. Braybrook, personal communication, July 14, 1995).

*Highly degraded cellulosic materials* are residual materials resulting from severe oxidation, hydrolysis, or mechanical damage, that are not identifiable using conventional methods.

*Material* is the term used in the thesis to refer to physical objects. Hence, *cellulosic materials* are physical objects composed of cellulose.

*Oxalic Acid* is a dicarboxylic acid, $(COOH)_2$, which is formed in plants during photosynthesis. It combines with calcium to form calcium oxalate crystal deposits in plants (Kausch, 1983).

*Phytolith* is a term that seems to have been universally accepted by archaeologists and paleoecologists in referring to silica, and in cases to calcium oxalate as well. Botanical literature discusses calcium oxalate and silica without using the term phytolith. In this thesis, the term phytolith refers to both calcium oxalate, hydrated silicon dioxide, and other mineral inclusions in plant tissues (see *Nomenclature* in the thesis *Literature Review*).

*Plasma Ashing* (see *Cold Plasma Ashing*)

*Monosilicic Acid*, $Si(OH)_4$, is absorbed by plants from the soil, and forms hydrated silicon dioxide, $SiO_2 \cdot nH_2O$ (Jones & Handreck, 1967).

*Silicon Dioxide, Hydrated,* $SiO_2\,nH_2O$, is an amorphous form of silica found in and around plant cells, formed from monosilicic acid (Jones & Handreck, 1967).

*Thermal Ashing* is an ashing method accomplished by heat oxidation, or combustion.

# CHAPTER 2: REVIEW OF THE LITERATURE

## Introduction

A review of the literature was the first step towards addressing the research problem, and it involved a gathering and review of information from several disciplines. The concepts that were explored included highly degraded cellulosic materials identification; cellulosic materials conservation; plant anatomy; mechanisms of calcium oxalate and silica deposition in plants; phytoliths; phytolith history; phytolith analysis; and the limitations of phytolith analysis.

## Plants

Before proceeding with the discussion of calcification and silicification in plants an outline of plant anatomy and metabolic processes is necessary. The anatomy of a plant can determine the morphology of silicon dioxide deposits, and the metabolic activity of a plant determines the patterns of deposition of calcium compounds and silicon dioxide. Also, knowledge of metabolic processes assists one in comprehending the movement of ions into and through the plant and, therefore, where the points of deposition may occur. The chemical reactions that result in the formation of hydrated silicon dioxide, calcium oxalate and other mineral compounds must also be considered. The chemicals present in soils can determine the qualities and quantities of mineral deposits in plants. As well, the circumstances necessary for the formation of calcium oxalate and silicon dioxide are specific to certain locations in plants due to the chemical nature of the metabolic activities taking place.

### Plant Organs

The kingdom Plantae consists of the vascular plants (those that conduct water and nutrients) and the non-vascular plants. The vascular plants are further subdivided into vascular cryptograms (with hidden reproduction, e.g. ferns and equisetum) and the vascular seed plants, the spermatophytes (Mauseth, 1988). Materials used in textile artifacts are from both vascular and non-vascular plants, however, the cellulosic materials that were used in the research supported by this discussion came from vascular seed plants. Therefore, this report, discusses vascular seed plant tissues and organs.

A vascular seed plant, as an adult, is characterized by the presence of three organs. The roots serve to anchor the plant into the ground and to bring water and inorganic ions to the developing tissues. The stem serves to elevate the major photosynthetic portion of the plant, the leaves, towards the photosynthetic energy source, the sun (Raven, Evert & Curtis, 1981). The three organs are interconnected by an efficient vascular system for the transport of water from the soil and nutrients from the photosynthetic portions. All or parts of each of the plant organs (roots, stems and leaves) are used within textile artifacts, however, this study focuses on the stem and leaf organs.

### Plant Cells

Each of the organs is composed of cells that have differentiated to form three tissue systems -- vascular, ground, and dermal (Alberts et al, 1983). Every cell of a vascular plant is one of three different kinds of cell structure, or differentiated versions of one or the other of the three kinds of cells. Every cell is composed of cytoplasm, contained in a plasmalemma, or plasma membrane, and surrounded by cell wall. The cell wall is composed of microfibrils, which are collections of long-chain cellulose molecules extruded from rosettes that move within the surface of the plasmalemma (Mauseth, 1988, p. 36). Thus, the cell wall is exterior to the plasmalemma from the cytoplasm, and is built-up on its proximal, or inner surface. The three kinds of cell structures are different from one another in the quantity, arrangement, and chemical nature of cellulose in their walls. Parenchyma cells have a thin primary cell wall; sclerenchyma has several layers of secondary cell wall; and, collenchyma has unevenly thickened secondary wall. The secondary cell wall material tends to be more rigid than the primary cell wall due to the alternating nature of the deposition of cellulose. The cellulose in the secondary cell wall is deposited in layers, with the cellulose fibrils of the adjacent layers oriented at a degree of rotation relative to the layer below. Within and between the layers is a matrix of hemicellulose, pectic substances, proteins, and lignin.

The three kinds of cells differentiate to form cells with specialized functions, such as the differentiated parenchyma of the epidermis found on each organ; the parenchyma called "chlorenchyma," which contains the chlorophyll necessary for photosynthesis; the highly elongated, heavily thickened secondary walled sclerenchyma, called "fibres"; or the modified parenchyma and sclerenchyma, which make up the phloem (columns or rows of nutrient passage cells), and xylem (columns or rows of water passage cells), of the vascular system.

In addition to understanding how differentiation of plant cells is possible and takes place, there are three important facts with regards to plant cells in general. The first is that plant cells contain vacuoles, or intracellular spaces that serve a variety of purposes, including the displacement of the cytoplasm to within a layer that is next to the plasmalemma. These vacuoles, in most cases, are the site of primary deposition of calcium oxalate, some silicon dioxide, and other mineral compounds, such as calcium carbonate (Arnott, 1974). The second note is that each cell is not merrily existing on its own, in fact, every cell is in communication with its neighboring cells through the intricate system of tubules of the endoplasmic reticulum through the plasmodesmata (discontinuities in the plasmalemma and cell wall). The interconnectivity between the cells supports the belief that mineral formation is not a random activity but, rather, a necessary function of the developing plant. The third note is that some cells within plants are no longer living -- they serve a structural role (fibres), protective roles (bark), or, in the case of vessel members of the xylem, a conductive role. The chemical requirements of living cells are not an issue for dead cells and, therefore, the patterns of deposition of calcium oxalate and silicon dioxide in and around dead cells differs from the way it is deposited in and around living cells. Lastly, it is important to note that living cells are in constant flux. The cytoplasm of the cells flows within the plasmalemma in patterns called "cytoplasmic streaming." Also, the changing seasons, temperatures, relative humidities, and chemical and light qualities all affect cell development and change (Esau, 1965; Mauseth, 1988). This last note indicates that the location of deposition within the plant and time in the life cycle of a plant when it is harvested may determine the quality, quantity and location of calcium oxalate and silicon dioxide in plant tissues.

### Inorganic Ion Transport In Plants

The mechanisms that result in the deposition of silicon dioxide, calcium oxalate, calcium carbonate and other minerals in plants on a gross scale are relative to the transport of water throughout the plant. On a fine scale the transport of ions involves mechanisms of further precision which can inhibit or encourage deposition -- such as an ion pump which is involved in the movement of calcium ions and oxalic acid across membranes (Pentecost, 1980). Further, the metabolic activity of plants results in the formation of both energy and products for reactions.

Water and inorganic ions from the soil are absorbed into the roots and then move throughout the plant via the tracheary elements, the xylem of the plant. The movement of the water through the xylem is driven by concentration gradients and capillary action, due to the cohesive nature of the $H_2O$ molecule and the relative humidity of soil and air surrounding the plant. This is considered to be passive movement. Some of the inorganic ions that eventually make up mineral deposits in the plant also move throughout the plant, passively with the water. Others are formed as a result of photosynthesis. Consequently, one should expect to find mineral deposits in plants at any points to which water is transported to, especially at points of photorespiration and transpiration (Jones & Handreck, 1967; Lawton, 1980; Mauseth, 1988).

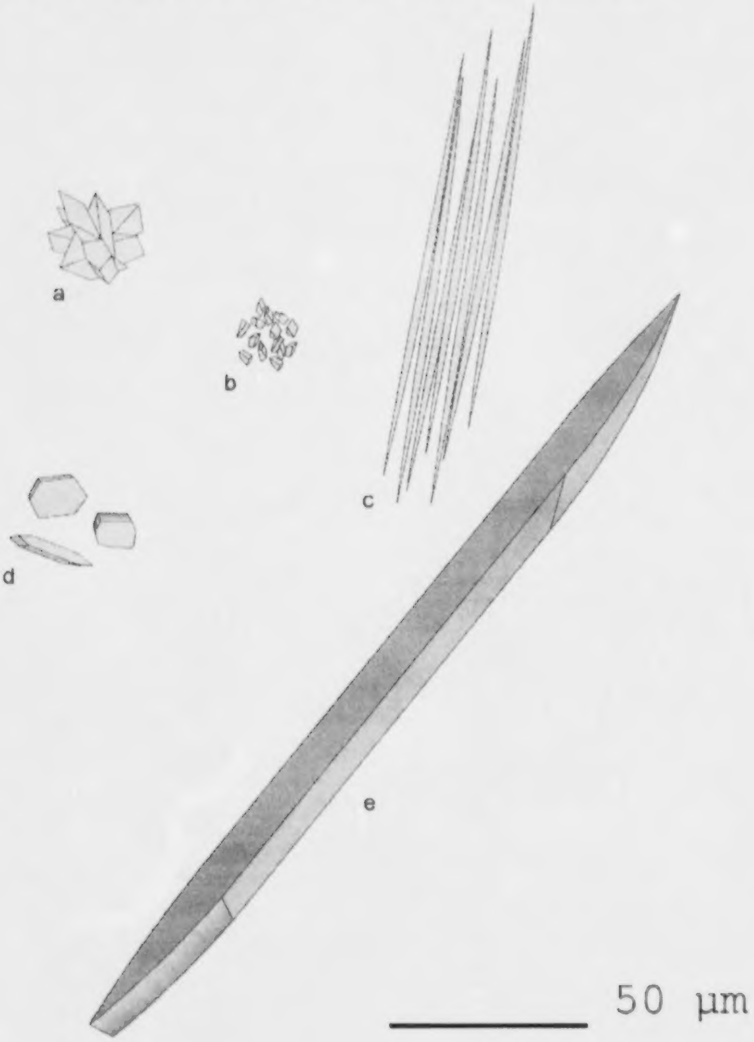
Calcium oxalate is an insoluble salt of the $Ca^{2+}$ cation and the $(COO^-)_2$ anion of oxalic acid. The movement of $Ca^{2+}$ ions throughout the plant is only understood in a general way, "but some aspects of its distribution are depicted in the calcium cycle" (Arnott, 1974, p. 56). Calcium is considered to be an important part of plant growth and metabolism (Arnott), and is thought to be deposited in mineral compounds to the highest degree in warmer periods of the year (Pentecost, 1980) and most predominantly during the photosynthetic periods of the day (Kausch, 1983). The oxalate anion, $(COO^-)_2$, is produced from the ionization of oxalic acid $(COOH)_2$, which is formed in plants "through at least five biochemical pathways" (Kausch, p.4). The most important pathways are products of photorespiration, glycolate and glyoxalate (a bypass product involving the Krebs cycle). The oxidation of these products, by an enzyme that has not been clearly identified, leads to the oxalic acid product (see Kausch for a thorough explanation of the processes). The ultimate production of calcium oxalate from its constituents appears to be "the result of a maintenance of ionic balance by the plant" (Kausch, p. 20), and may be reversible (Kausch). Ishii and Takiyama report that it "is developed to protect protoplasm from toxic action of oxalic acid which is an end product of metabolism" (1989, p. 423).

The crystals of calcium oxalate are considered to form primarily in the intracellular spaces of cells, and with one of five gross morphologies. Studies (Arnott, 1974; Franceschi & Horner, 1980; Kausch, 1983) have shown that crystal formation is initiated in young tissue (near meristematic tissues of all organs) in chambers that form within the vacuole for the specific purpose of containing the crystals. Kausch suggested that the formation of the crystals in young tissues was a response to the greater necessity to control ionic equilibrium. In contrast to calcium oxalate deposition, silicon dioxide seems to be in association with senescent cells rather than cells of young tissue (Lawton, 1980).

Calcium oxalate crystals are called by different names based on their morphology: crystal sand, raphide (or raphide files), styloid, druse, rhomboid (or prismatics), or conglomerate crystals, made up of a combination of one or more of the five different shapes (see Figure 1). The raphides are needle-shaped and occur in bundles; the styloids are broader, oblong shapes that occur singly; the druses are aggregates of platelets that make up a spherical star-like shape; and the rhomboids are pyramidal or bipyramidal in form, or cubic (Arnott, 1974; Esau, 1965; Ishii & Takiyama, 1989; Kausch, 1983; Mauseth, 1988).

The form that the crystals take on are thought to be somewhat dependent on water content. The general formula for calcium oxalate is $CaC_2O_4\ nH_2O$. As a monohydrate it is called whewlite, and as a dihydrate weddellite. The two compounds vary in their crystallization patterns. Whewlite tends to form monoclinic systems of

**Figure 1.** Forms of calcium oxalate ($CaC_2O_4$) crystals found in plants.



**Note.** Five forms of calcium oxalate crystals: a) druse, or cluster crystal; b) crystal sand; c) raphide crystals; d) rhomboid, or prismatic crystals; e) styloid crystal (see discussion in *Literature Review*).

crystals, and weddellite forms tetragonal systems. Raphides and styloids are considered to be formed from whewlite, and druses and prismatics from weddellite (Al-Rais, Myers, & Watson, 1971; Ishii, & Takiyama, 1989; Kausch, 1983).

Both silicon dioxide and calcium oxalate have been found in all plant organs. Kausch (1983) reported that the calcium oxalate crystallization process "occurs within highly specialized and complex cells called crystal idioblasts and is apparently under strict biological control" (p.1). He also noted that the "size, shape, location, and orientation of the idioblast, as well as the shape of the crystal produced within the idioblast, are considered to be characteristic for a given species" (p. 1). The specialized nature of calcium oxalate crystals has been investigated by many researchers in relation to anther connective tissue (Horner & Wagner, 1992), leaf abscission zones (Grimson & Arnott, 1983), leaf mesophyll (Genua & Hillson, 1985), ray and axial parenchyma in woody plants (Taniguchi, Okamura, Harada, & Nakato, 1984), the sclereids of Nymphaea tetragona, water lily (Long & Huang, 1992), and gramineae, grasses (Lersten, 1983).

Silicon dioxide is deposited in and around plant cells as amorphous hydrated silica, $SiO_2 \cdot nH_2O$, from monosilicic acid, $Si(OH)_4$, which is absorbed by the plant from the soil (Jones & Handreck, 1967). The extent, location and mechanisms of deposition, have been studied by many researchers. The silicification patterns and morphologies have been studied to a great extent both by botanists and by archaeologists involved in phytolith analysis of paleoenvironments. However, there is some discrepancy in the understanding of the role of silicon dioxide deposits in plants. Certain functions of silicon dioxide in plant cells have been accepted, such as the need for embrittlement in the stinging hairs of Urtica dioica, stinging nettle (Esau, 1965; Mauseth, 1988; Thurston, 1974). However, there is debate as to whether silicon dioxide plays a role in support, contraction of cells, or light transmission to lower tiers of chlorenchyma cells (Bombin, 1984; Jones & Handreck, 1967; Sangster & Parry, 1981).

Many factors affect the content of silicon dioxide in plants. The soil in which a plant grows, the species of plant, transpiration, and nutrient supply all affect the deposition of silicon dioxide to varying degrees. The presence of monosilicic acid in soils will directly affect the deposition of silicon dioxide in plants. However, the uptake of monosilicic acid can be hindered by the presence of sequestering metal ions in the soil or the pH of the soil. The solubility of silica is independent of pH ranging from 2 to 9. Certain species tend to accumulate silicon dioxide to a greater degree than other species. "It is generally accepted that Gramineae contain 10 to 20 times the concentration of silica found in legumes and other dicotyledons" (Jones & Handreck, 1967, p. 112). Jones and Handreck suggested that the differential may be "attributed to an exclusion of monosilicic acid from the transpiration stream, either within the root or at its external surface" (p. 115).

Certain plant species are thought to lack silicon dioxide. This conclusion may be the result of studies that compare deposits in only one plant organ. Indeed, silicon dioxide may occur in organs other than those being studied. Transpiration rates in different plants may also affect silicon dioxide deposition. In an experiment in which the relative humidity in growing environments was varied, it was determined that when transpiration rates were lower, the concentration of silicon dioxide decreased (Jones & Handreck, 1967). The transpiration rate is thought to be controlled somewhat by the nutrient content of the soil. The concentration of minerals can also affect the uptake of monosilicic acid, such that the other ions will lower the potential concentration of monosilicic acid in ppm (Jones & Handreck).

The distribution of silicon dioxide in plants seems dependent on species. In some species it may be found in roots, in others the secondary wood contains the deposits, and in yet others, the leaves contain the majority. Though not all plant species have been studied, the tendency is for silicon dioxide to be concentrated at sites adjacent to the transpiration stream. Three categories of deposits have been suggested: (a) cell lumen in-fills, (b) cell wall deposits, and (c) intercellular deposits. In cases where the lumen is filled with silicon dioxide the deposit will be characteristically shaped and may be of taxonomic value. In cases where the deposits are associated with the cuticular layer of the epidermis they form in sheets and take on the features of the epidermal cell layer, which, for some species, may provide aid in taxonomy (Sangster & Parry, 1981).

The Gramineae (grasses) have been studied extensively due to the characteristic ontogeny of leaf development, and, as a result, due to the abundance of silicon dioxide found in their leaves. The development of a grass leaf begins with all cells appearing approximately the same. By the time the leaf has finished growing, it consists of a pattern of long and short cells, with the short cells being further differentiated into cork and silica cells. In a study of the plasmodesmatal connections the following was observed:

(a) connections between long cells,
(b) connections between cork and silica cells,
(c) no connections between long and silica cells, and
(d) connections between cork and long cells.

The patterning is said to suggest a "close correlation between structure and function" (Lawton, 1980, p. 161).

Grass leaves can also be silicified in xylem vessels and in marginal sclerenchyma cells, and deposits can be found in seeds (Lanning & Eleuterius, 1991). The great concentrations of deposits in the leaves of Grasses are thought to have a deterrent affect on insects and disease. However, their presence has also been connected with rates of esophageal cancer in an area in the Lin Xian province in northern China. The sharp fragments of silicon dioxide have been found in mucosa of cancer patients. The fragments have also been connected to the irritating and toxic reaction of skin to contact with certain plants (Lanning & Eleuterius, 1987). Calcium oxalate crystals have been similarly connected with respiratory problems of workers that have breathed in cotton dust from the commercial cotton mill workrooms (Goynes, Ingber, & Bernie, 1984).

### Summary

An understanding of plant anatomy and metabolism is necessary for any researcher that is attempting taxonomy of plant materials used in artifacts. This is particularly true when one is proposing to identify the plant by a characteristic that is a result of metabolic activity and that takes its morphology from plant anatomy. Phytolith analysis propounds the study of a characteristic of plants that persists through degradation of the organic portions of the plant. Phytolith analysis, then, involves the study of assemblages of silicon dioxide and calcium oxalate deposits in plants. Since the assemblages and patterns of deposits in and around plant cells seems to vary from species to species then taxonomy based on this form of analysis would be possible.

### Cellulose Chemistry

A knowledge of the chemistry of cellulose is important in understanding the degradation that takes place in plant materials of artifacts. A plant is no longer living once it has been rendered for use in textiles or textile related artifacts. In most cases, the cytoplasmic contents are not only not functioning, but they have also been "lost," i.e. digested or incorporated by the plant while it was still living, or degraded once that plant has died. Thus, the major component of textile materials is the cell wall, which, in most cases, is composed of cellulose. Cellulose is "a linear polymer constructed from the condensation of ß-glucose molecules linked together through the 1 and 4 positions" (Peters, 1963, p. 181). The molecules of cellulose, with multiple hydroxyl groups, achieve close proximity to one another and form hydrogen bonds, and, consequently, cellulose microfibrils, macrofibrils, and cell wall. In areas where the cellulose molecules are highly oriented, crystalline micelles can form. The crystalline sections intersperse with amorphous regions (Jakes and Sibley, 1983; Kronkright, 1990; Mauseth, 1988, p. 35).

Polymerized glucose units can be broken at the 1,4-ß-glucosidic link by hydrolysis, the breaking of the bond and addition of a water molecule, in an acid catalyzed reaction. "In advanced stages of hydrolytic deterioration, the fibre degrades to a powder composed of cellobiose and mixed oligosaccarides" (Jakes and Sibley, 1983, p. 32). In a hydrolysis reaction energy is released. The chemical bonding energy of the products of hydrolysis is less than that of the original long-chain cellulose molecule. Microbiological degradation of cellulose can also occur. As with other forms of degradation, the areas which are first attacked are the amorphous regions of the cellulose. As cellulose is degraded, it becomes more crystalline; this is known by measuring the density of degraded cellulose. This fact is important to note, since the remains of cellulosic textiles will likely have more crystalline regions intact and more amorphous regions destroyed, and therefore the reactivity of the cellulose once it is degraded will be different than when it is intact (see Kronkright, 1990, for a complete discussion of cellulose degradation).

## Phytolith Analysis

The following discussion reviews the literature which references phytolith nomenclature, phytolith history, phytolith distillation methods, phytolith classification systems, the interpretation of phytolith assemblages, and the limitations of phytolith analysis.

### Phytolith Nomenclature

Calcium oxalate and silica deposits in plants are referred to in the literature by a relatively limited number of names. In general they are grouped with other products of metabolism as ergastic substances (Mauseth, 1988; Metcalfe, 1963). A few authors refer to both substances as phytoliths (Baker, 1959; Cummings, 1992; Florian, 1990; Jones & Bryant, 1992), however, calcium oxalate as crystals seems to be ubiquitous in the literature. As aforementioned, calcium oxalate crystals are also called by different names based on their morphology: crystal sand, raphide (or raphide files), styloid, druse, rhomboid ( or prismatics), or conglomerate crystals, made up of a combination of one or more of the five different shapes (Arnott, 1974; Esau, 1965, figure 2.6; Kausch, 1983; Mauseth, 1988). The terms idioblast, or crystal idioblast are sometimes used by botanists to refer to the cells which contain the crystals (Cronquist, 1981; Esau; Kausch & Horner, 1983; Mauseth; Wheeler, 1979). Cystolith and lithocyst are two terms which are used in reference to calcified plant cells; cystolith refers to the content of calcium compound and lithocyst refers to the cell itself (Esau; Mauseth; Nakamura, 1969). There was no literature found where silica deposits are referred to as crystals.

Silica deposits are sometimes called plant opal, opal phytoliths, opaline silica, or grass opal (Pearsall & Piperno, 1993; Piperno, 1988). The reference to the term opal comes from the chemical and physical similarity of the silica deposits found in plants and precious opal. Both opal and silica in plants are amorphous forms of silicon dioxide, or silica ($SiO_2$), which is the same compound that makes up quartz (crystalline form of $SiO_2$) and chalcedony (pseudocrystalline form of $SiO_2$). Despite the fact that precious opal and plant silica are both amorphous forms of silica, "there exists a very important difference between them. The phytoliths possess a great specific surface whereas opals have small specific surfaces" (Peinemann, Tschapek, & Grassi, 1970, pp. 131-132). The pitted nature of the plant silica that leads to the great specific surface area can be seen in scanning electron micrographs (SEM) (Mulholland, 1989, Figure 1; Pearsall & Piperno, Figure 4).

Silica deposits in nature in general are termed bioliths (Bombin, 1984; Piperno, 1988; Zucol, 1992) and in plants are called biogenic silica, silica bodies (Piperno), or silica corpuscles (Bombin). In early phytolith literature the silica deposits are named for their morphology: circular, elliptical, oblong, cross, dumbbell, crenate, elongate, etc. (Metcalfe, 1963; Twiss, Suess, & Smith, 1969). For over a decade the citations followed the nomenclature that had been established by the early researchers (Bombin). In the 1970s and early 1980s, possibly following the progress of Rovner (1971, 1972, 1975), the opinions of Bombin, who refuted the Twiss, Suess, and Smith approach, or the work of Brown (1984), who provides his own "fresh start" (p. 6), the general momentum of the field was towards "a fresh approach to the method of phytolith analysis, within the framework of plant anatomy, ontogeny and new theory" (Bombin, p. 5). Thus, later literature refers to silica deposits using names that reference the various cells of plant anatomy, epidermal including trichomes, stomata, and bulliform cells; hypodermal; mesophyll; sclerenchymous; and vascular (Piperno, 1988).

An issue of nomenclature that will be discussed in the phytolith distillation section of this review is the notion that the term phytolith should be applied to both silica deposits and calcium oxalate deposits. While there is inconsistency of action on whether or not calcium oxalate crystals should be considered during phytolith analysis, Bombin claims that the term phytolith should be reserved for silica deposits "on natural and practical grounds" (1984, p. 11). Subsequent literature that cites Bombin does not support or refute his argument, or rather, statement, since he does not support or explain what he really means by "natural and practical."

The term phytolith translates literally from Greek to read plant stone (Bozarth, 1993). Dunn (1983) and Baker (1959) suggest that the term was applied by Ruprecht in 1866, while others (Bombin, 1984; Zucol 1992) credit Ehrenberg with coining the term phytolitharia, which translates to English from German as phytolith, in 1841. The term phytolith seems to have been universally accepted by archaeologists and paleoecologists in referring to silica, and in cases to calcium oxalate as well. Botanical literature discusses calcium oxalate and silica without using the term phytolith. For the purpose of my research, when the term phytolith is used it refers to calcium oxalate, silicon dioxide (silica), and other mineral inclusions. Most references to substances is by their chemical names, such as calcium oxalate and silicon dioxide (or silica), and their morphological characteristics.

### History

The history of phytolith analysis is said to span approximately 150 years and to be punctuated with great periods of dormancy. The perspective of archaeologists and paleoecologists purports that phytolith analysis was concurrently introduced with and then superseded by pollen and diatom analysis (Bombin, 1984; Pearsall and Piperno, 1993; Rovner, 1971). However, the study of calcium oxalate and silicon dioxide in plants has been a continuous, though modest element of the field of Botany (Lanning, Ponnaiya, & Crumpton, 1958; Lersten, 1983; Parry & Smithson, 1958, 1962) since its 19th Century introduction.

By the end of the 18th Century there was an awareness of silica in plants. Bombin (1984) discovered Struve, an early botanist commenting on the solubility of plant silica in hot potassium hydroxide, though he found it to be insoluble in acids. An early German researcher, Ehrenberg, originally thought that the silica he found was from an animal living in the plant. In fact he must have been correct in part. The illustration in Bombin's thesis of one of Ehrenberg's plates does appear to have some drawings of diatoms. The illustration by Ehrenberg is considered an attempt by Ehrenberg to classify the forms that he found (Bombin). Subsequent phytolith studies have all attempted to refine the classification systems. Early studies by botanists did not focus on phytoliths in soils. Bombin suggests that it was in the 1920's in Argentina that the study of fossil silica was begun, but that the findings were not widely publicized because they were written is Spanish. Though silicification and calcification in plants was being studied by botanists on a sporadic basis in the first half of the 20th century, the study of plant silica in soils observed a hiatus through the 1930s, 1940s, and 1950s (Bombin).

In response to the evolving interest in the relationship of people to their environment in prehistory, a new tool to study the paleobotanical environment became necessary. Rovner (1971) explains how the need for an analytical tool other than pollen analysis was needed, a tool which studied plant remains that persisted beyond degradation of the organic portions of the plants. At the same time the field of archaeology required the introduction of an interdisciplinary approach -- to draw on the natural sciences. There is a rash of literature from the late 50s and into the 60s, by soil scientists and botanists, about silica and about calcium oxalate. In 1969 the paper by Twiss, Suess, and Smith was published. It was a first modern attempt to classify phytoliths. At first, these classifications were used by other researchers. Eventually, however, the limitations of the classification system became apparent. Bombin (1984) points out how the major problems were a) that Twiss, Suess, & Smith had presented material in a review that was taken to be a primary source and b) that the nomenclature of the shapes was not based on plant anatomy. By 1988 there was a whole new wave of researchers occupied with systematics, including classification, distillation procedures and nomenclature, for the most part, of course, dealing with plant silica. As each new wave of researchers advances, the field becomes more systematized. Research is currently ongoing in at least three places in the United States, at Ohio State University, Columbus, at the University of Pennsylvania, Philadelphia, and at the University of Missouri-Columbia (Jakes, 1994; Pearsall, 1994a; Piperno, 1994).

The contemporary use of phytolith analysis covers a broad spectrum of disciplines. Bombin (1984) and others (Pearsall & Piperno, 1993; Piperno, 1988; Rapp & Mulholland, 1992) suggested a list of the possible interdisciplinary uses of phytolith analysis including paleobotanical studies, paleoenvironmental studies, complementing pollen analysis, reconstructing past diets, the involvement of diet in pathologies, archaeological reconstructions, soil genesis studies, world distribution of C3 and C4 grasses, dating of occluded radiocarbons, and forensics. For my part, I wish to add identification of highly degraded cellulosic materials to the list, which may be covered by "archaeological reconstructions" or "forensics," but that may need to be qualified as a separate concern.

### Distillation

The removal for analysis of the deposits of calcium oxalate or silicon dioxide that form in plants as they grow, or phytolith distillation or extraction (Pearsall and Piperno, 1993) can be accomplished by a variety of means. The techniques that are discussed in the majority of the "phytolith analysis" literature are specific to distilling silicon dioxide from soils for analysis, and from plants in the creation of reference collections. As Ollendorf, Mulholland, and Rapp (1988) suggested, most recent work in the area of phytolith analysis is focused on studying silicon dioxide in soils. Writers in the field (Pearsall & Piperno, 1993; Piperno, 1988; Rovner, 1988) acknowledge in one sentence that plants do form inorganic compounds other than silica, but then they proceed to discuss the distillation, classification and interpretation of only silicon dioxide. The techniques that are used to remove the silicon dioxide phytoliths from soils includes the washing of the soil, the acid dissolution of organic materials in the soil, and then the separation of the phytoliths from soil in heavy liquid. Rovner's (1971, 1972) technique is the one cited most frequently throughout the 1980s. His process includes an overnight soak in distilled water (silica is found in tap water) and sodium metaphosphate (Calgon ®) to disaggregate the soil, followed by the removal of organics and carbonates in hydrochloric acid. The solution is then centrifuged and separated using a heavy liquid created "by adding tetrabromoethane to absolute ethyl alcohol until a specific gravity of 2.3 is reached" (p.347, 1971). The supernatant taken from the surface is then centrifuged and then the phytoliths are settled to the bottom of the centrifuge tubes using ethyl alcohol to reduce the specific gravity of the liquid. In 1992 Rapp and Mulholland outlined the refinements of the same technique which amounted to the screening of the samples, the substitution of warm hydrogen peroxide to remove organic compounds, and the suggestion of heavy liquids other than tetrabromoethane which is pointed out to be carcinogenic. The heavy liquids which are now most commonly used are zinc bromide or potassium iodide with cadmium iodide (Pearsall, 1994b).

The creation of reference collections of silicon dioxide phytoliths from known living plant specimens involves either the maceration of plant specimens followed by wet oxidation, wet ashing in acids, or the heating of the specimens in a furnace, dry ashing. Botanists have also worked with combinations of techniques that result in an accurate understanding of the positioning of the silicon dioxide within the overall anatomy of the plant. "By combining the facts learned from the different techniques it is possible to visualize in a three-dimensional sense the positions of the opals within the structure of the leaf" (Parry and Smithson, 1958, p. 544). Parry and Smithson's work included clearing plant materials (rendering the plant materials transparent) and serial sectioning. The clearing of plant materials was done using successive applications of ammonia and hydrogen peroxide, or chromic acid (carcinogenic), and the sectioning of plant materials was mostly done at 10 microns, though some needed to be thicker to accommodate larger deposits. One other technique for detecting silicon dioxide in plant specimens is through the use of histochemical (staining) reactions (Dayanandan, Kaufman, & Franklin, 1983). The staining of the phytoliths while still in cleared leaf sections results in being able to see them more clearly than if they were distilled and placed in a mountant.

However, the technique, which was described in 1983, does not seem to have been used recently.

Thermal ashing or chemical digestion of plants tends to produce a bulk yield. The dry ash method has met with mixed reviews. It can be done either in a muffle furnace in crucibles (AATCC Test Method 78, 1993; Catling & Grayson, 1982), or between two glass microscope slides (Lanning, Ponnaiya, & Crumpton, 1958), which results in a retention of positioning of the phytoliths as they would have been in the plant. In either case, Rovner (1971) claimed in his review of the furnace ashing technique that there can be artifacts of the process; he pointed out that the heat of the furnace can cause the phytoliths to distort and to fuse together. Rovner cited instances where the surface porosity of the phytoliths is altered due to the heat of dry ashing. The claims that Rovner made were refuted by Piperno (1988) who stated that at temperatures not above 500°C for one hour "opaline silica does not change to other forms of silica and morphology remains unaffected" (p. 126). However, Jones and Bryant (1992) again reported the distortion of silica and destruction of calcium oxalate when using the dry ashing technique. The incineration is followed by a series of acid baths, 10% hydrochloric acid, concentrated nitric acid, 10% hydrochloric acid, and benzene, and drying in acetone, or the ash is used to create a spodogram (from the Greek word spodos, meaning ash) (see Piperno, 1988, pp. 126-127 for technique). Wet ashing involves chemical dissolution, an acid bath, and drying. Piperno outlines the many wet ashing methods that have been and are being used. Schulze's solution (3:1; $HNO_3$:$KClO_3$, or $HNO_3$:$NaClO_3$) was borrowed by Rovner (1971) from the field of pollen analysis, and is still being used by most researchers, though chromic acid (carcinogenic) is also still being used (Ball, Brotherson, & Gardner, 1993).

One step that is common to all the methods used in the distillation of silicon dioxide phytoliths is the use of acids. The problem with the use of acids is that they will dissolve calcium oxalate (Al-Rais, Myers, & Watson, 1971). For the purposes of studying highly degraded cellulosic materials I believe that the retention of both silicon dioxide and calcium oxalate will be significant. Researchers that confer with me on this issue are also looking to use techniques that retain not only the silicon dioxide, but also the calcium oxalate (Cummings, 1992; Jones & Bryant, 1992). Recent archeological literature continues to focus only on silicon dioxide, but changes may be in process. Cummings comments on the "importance of exploring the use of methods that retain as much as possible of the crystal as well as the opal phytolith data" ( p. 190). Jones and Bryant writing about the techniques of silicon dioxide phytolith distillation stated that the "extraction techniques have two things in common. They are all dangerous and all are designed for the extraction of silica based phytoliths" (p. 221). Cummings suggested the use of hydrogen peroxide, on occasion with a catalyst, and Jones and Bryant used hydrogen peroxide with potassium dichromate (possibly the catalyst Cummings spoke of but didn't identify). The hydrogen peroxide (a 30% concentration) is used in a fume hood at room temperature for two minutes, then potassium dichromate crystals are added. In tests that were conducted the oxidation process rarely took more than twenty minutes (Jones & Bryant).

Cold plasma ashing is one final method of distillation that has been used to successfully study phytoliths. The researchers (Umemoto, Hutoh, and Hozumi, 1973) who used cold plasma ashing reported the following:

> When active oxygen plasma excited by high-frequency energy is applied to the plant tissue, organic material in the tissue is gently removed at ordinary temperatures, and the residual ash is left behind without destruction of the mineral microstructure in the original tissue matrix. [The researchers ]...noted particularly the characteristic presence of crystals of calcium oxalate, calcium carbonate and silicon bodies in the ashed tissues, together with their specific shapes and distribution patterns, and [they]... suggested that the identification of the plant might be possible by comparing the shape and distribution patterns of crystals in the ashed tissue. (p. 301)

Cold plasma ashing or one of the calcium oxalate "friendly" wet ashing techniques were the first to be considered appropriate for my research.

Catling and Grayson (1982) report having used ashing in crucibles at 600°C and then creating spodograms from the ash. Based on the discussion of dry ashing earlier in this review, one can imagine that the crystals that were distilled by Catling and Grayson may have been distorted. They did, however, publish the summary of the calcium oxalate that one could expect to find associated with a variety of fibres of Western economics (see Table 1). Ash analysis is also the method used by Schaffer (1981a) in studying ethnographic textile collections at the National Museum of Man, Ottawa, however, she does not use the muffle furnace method, but, rather, ashes in a crucible over an open flame. Schaffer provides a summary (see Schaffer, 1981a, Table 1) of where and what type of silicon dioxide and calcium oxalate phytoliths might be found in or adhering to textiles. However, she disclaims the results because, "in processing, fibres lose some of the crystalline substances, so that absence cannot be regarded as conclusive" (p.124). Körber -Grohne (1991) used hydrogen peroxide and aqueous ammonia ($NH_4OH$) on charred linen textiles in order to clear any fibres that were still intact. Körber-Grohne does not report any phytoliths on linen, but she does discuss the presence of silica in sacking materials from the archaeological collections from the Alpine region to Finland.

Once the phytoliths have been distilled, it is necessary to prepare them for examination under light microscope, SEM, Transmission Electron Microscope (TEM), or for EDXA. Though one research team has worked extensively with staining phytoliths for examination (Dayanandan, Kaufman, & Franklin, 1983), the majority do not use staining in the preparation of their slides. In fact, Bombin (1984) suggested that the stains made the phytoliths more difficult to distinguish. The mountants that are used can be permanent or fluid. Canada Balsam is used by many but considered to have a refractive index that can distort the view of the phytoliths (Bombin, 1984; Dunn, 1983). "Hydrax, a medium developed for mounting diatoms which are the same chemical composition and refractory index as phytoliths, provides less distortion" (Dunn). Liquid mountants are used in situations where a three-dimensional characterization of the phytoliths is desired. The slides can be manipulated to move the phytoliths in the liquid mountant as they are being examined (Bombin). Preparation for SEM, TEM and EDXA follows standard procedures (Sangster & Parry, 1981).

### Classification Systems

The classification of phytoliths extracted from known plant specimens provides keys for the phytoliths distilled from unknown samples. Since the first attempts 150 years ago, many advances have been made. However, Rovner (1975) pointed out that " the problem of developing a usable phytolith taxonomy initially may seem comparable to the monumental task of developing the taxonomy of the plant kingdom in the first place or, perhaps, to the establishment of a pollen taxonomy" (p.134). In the last 20 years many plant families have been studied and keys have been developed. Most of the work that has been done is with grasses and in the area of the tropics. It is difficult to ascertain from the literature if the amount of research in a particular area is the result of researchers' interests or the probability of finding phytoliths in given taxa in specific geographic areas.

The classification systems that were used in the 1970s focused on individual phytolith morphology (Twiss, Suess, & Smith, 1969). Through the 1980s phytolith classification became more relevant to plant anatomy and began to consider "suites" or "assemblages" of phytoliths rather than individual shapes (Piperno, 1988). Understanding of the concept of a phytolith assemblage can be facilitated by using the analogy of the 26 letters of the English language. There are few letters and yet, depending on how they are assembled, they can compose a great variety of words. The contemporary approach to phytolith analysis is systematic in its distillation procedures and in the way in which the phytoliths are classified. Pearsall and Dinan (1992), reporting on the system at the University of Missouri-Columbia, wrote that their classification system is based on the hierarchical classification of shapes based on the plant tissue source, with lower levels of the hierarchies defining the phytolith's morphology in progressive stages (see Pearsall and Dinan, Figures 3.2, 3.4, & 3.6 to fully understand how the hierarchies function). The encoded hierarchies are complemented with a similarly hierarchical arrangement of phytolith photographs. The work of Pearsall and Dinan recognizes the significant contribution Brown made in 1984.

Brown's (1984) "fresh start" was an attempt to design a key for phytoliths that would be "expandable, generalizable to higher taxonomic levels, and separable by major plant parts" (p. 346). He outlined six sections that needed to be in a phytolith key:

1. Mounted voucher specimens.
2. Paper copies of specimens, indicating the location from where phytoliths had been taken.
3. Permanent slides of processed plant samples (Brown used ashing).
4. Micrographs of each microscope slide.
5. A shape analysis.
6. A manual of the morphological hierarchies to be used to in the analysis of unknown samples.

These six steps have been utilized by researchers in the last ten years. They provided a basis for the work of this thesis.

The classification of phytolith shapes using computer software is being explored by Russ and Rovner (1989) and Rovner and Russ (1992). Using stereology and computer-assisted image analysis, and a formula that compiles the description of the phytolith as read by the computer, they are able to classify the phytolith. Pearsall and Piperno (1993) have tested Russ and Rovner's work using their own methods and have found no advantage to the computer assisted descriptions. Russ and Rovner suggested that the computer process can assist in the processing of large volumes of data, a task that can be tedious and have a high fatigue factor. Many fields of study use computers effectively to process large volumes of data. Likely the conclusions made by Russ and Rovner about computer analysis are accurate. Computers are an appropriate "work horse" for the tedious job of describing the phytolith data, as well as being an efficient tool for storing the collected data. Data that are collected and stored digitally can be revisited and reviewed more readily that data that are stored by other means.

### **Interpretation of Phytolith Assemblages**

The interpretation of the phytoliths that have been distilled from unknown samples involves comparison with the known classified samples. In theory, with sufficiently developed keys, a researcher should be able to identify a plant family, and in instances, genus, or species. There appear to be only two instances in the literature where the analysis of silicon dioxide phytoliths has established the preexistence of a species (Ollendorf, 1987; Piperno and Pearsall, 1993). Pearsall and Piperno and other researchers (Bozarth, 1993; Mulholland, Rapp, Ollendorf, & Regal, 1990; Umlauf, 1993) have studied Zea mays (corn) at the species level in paleoenvironments by using phytolith analysis. The identification of a silicon dioxide phytolith that is characteristic to Zea mays has been the subject of much study and a little contradiction. Calcium oxalate phytolith studies to date are limited to working with reference collections (Cummings, 1992; Jones & Bryant, 1992) even though there is an equivalent wealth of botanical literature on the subjects of calcium oxalate occurrence and silicon dioxide occurrence. Of course, one must remember that phytolith analysis, for the most part to date, is the distillation, classification and interpretation of only silicon dioxide phytoliths. Perhaps the systematics of phytolith analysis will begin to incorporate calcium oxalate phytoliths as well as silicon dioxide phytoliths.

Dunn (1983) conducted research to corroborate the interpretive work that had been done on Zea mays' "cross-shaped" and distinguishably large leaf blade silicon dioxide phytoliths. In the results section of her research report she lamented that her evidence does not favour support of earlier findings. However, a review of her data in comparison to the size variations recorded by earlier researchers does suggest that Zea mays is distinguishable.

Dunn reported finding the following size variations:

| | | |
|---|---|---|
| 1. | Zea mays L. | 12.5 - 25.0 microns. |
| 2. | Sorghum halepense L. | 12.5 - 20.0 microns. |
| 3. | Tripsacum datyloides L | 12.5 - 17.5 microns. |
| 4. | Zea mexicana (Schrad.) Kuntze | 7.5 - 12.5 microns. |

Indeed, the size variations that are proposed for maize and wild grasses suggest that Zea mays, and not any of the wild grasses, fall in the large and extra large categories, 16.03 - 20.56 microns and 20.61 - 25.19 microns, respectively. One can clearly see that although Dunn found a wild grass, Sorghum, in the large category, she found that only Zea mays fell in the extra large category. One should also note that Dunn admits at the beginning of her research report that her data base was limited.

Doolittle and Frederick (1991) conducted research to disprove that maize had distinctive silicon dioxide phytoliths. They conclude that "the use of cross-shaped phytoliths as indicators of prehistoric maize cultivation is seriously questioned" (p.183). They collected samples every second week beginning 14 days after germination and chose a chemical method of extraction. They perhaps misunderstood that in maize cultivation, the plants would not be harvested in successive two week periods -- this may have affected their results. Also their reasoning behind choosing a chemical method is questionable. They suggested that botanical studies utilize chemical methods and that archaeological studies use ashing. In fact, to read a few papers in each area one quickly sees that the reverse is true. The method they used included clearing with (50:50) diluted bleach:deionized water, followed by dehydration, mounting in Canada Balsam, and examination under 200x light microscopy. They conclude that no such cross-shape phytoliths exist, though they do say that even if they did exist, they would be indistinguishable from other species that contain them since they exist in many species. Perhaps they are speaking of redundancy.

The interpretation of silicon dioxide phytolith assemblages has contributed to determining family population densities, and the shifts in forestation versus agricultural plantings or wild grass horizons (Piperno, 1988; Rovner, 1988). The conclusions that have been achieved may be a reflection of the desired result. Paleoethnobotanists are focused on looking for the presence or absence of domesticated plants (Piperno, 1988, p. 136), thus they collect and process data with that goal in mind. In a 1988 review article Rovner presents a few more precise examples of the interpretation of phytolith assemblages. For example, he explains how a post-Pleistocene bison kill site yielded phytolith data that enabled interpretation of the buffalo's diet, and thus from where it had traveled, and the fact that dung had been burned in the hearth fire, and not wood. The phytoliths were also radiocarbon dated to provide corroborating evidence for the date of the site.

Interpretation also involves the use of statistics. Possible quantitative methods and statistical analyses in the study of opal phytoliths have been examined. Histograms are popular to view trends, but more precise constrained classification techniques (CCT), cluster analysis, and correspondence analysis have been conducted on data. CCT and cluster analysis are used to compare and contrast sample groups. As the name suggest, CCT requires more defined sets of data, but in the end presents dissimilarities as well as similarities within a group of sets. CCT seems appropriate for phytolith assemblage interpretation (Powers-Jones & Padmore, 1993).

### Limitations

Every process, artistic or scientific, has limitations. For phytolith analysis, as with other processes, the researcher can be one limitation -- the results being looked for, the understanding of methodologies, the procedural accuracy, and the ability to draw on colleagues and interdisciplinary knowledge. Phytolith analysis is also limited by historical factors that have already been discussed in previous sections of this review -- a result of the methods of extraction and classification. In his 1988 article Rovner addressed some of the practical limitations of phytolith analysis. The two main concepts that need to be addressed are those of multiplicity and redundancy (Rapp & Mulholland, 1992; Rovner, 1971). Again I draw on the analogy of the 26 letter English alphabet to present a concept. The letter "a," for instance, is found in many words; that is redundancy. In instances, the same letter, "t," for example, can be found more than once in a single word; that is multiplicity. A single plant will have a collection of like shaped phytoliths, or a multiplicity of phytoliths all similarly shaped. And, the phytolith found in one plant can be redundant across a family, and even throughout the plant kingdom. Redundancy is being reduced through the use of stereological and image analysis techniques (Rovner & Russ, 1992).

The factors of redundancy and multiplicity could be seen as limiting, but, in fact, they can also be a factor in making classification possible (imagine if there were more than 26 letters in the English alphabet). And, with classification systems in place, the interpretation becomes even more possible. Rovner suggested that the fact of multiplicity and redundancy can be used to advantage in establishing indicator species; such as with the 26 letter alphabet, which indicates that one is dealing with the English language, and therefore one knows what "word assemblages" to look for.

Another practical limitation is the fact that even though phytoliths are inorganic and therefore not readily degradable they do wash away, blow away, and shatter. Silicon dioxide phytoliths are susceptible to chemical reactivity with metal ions in soils and they will dissolve in environments with a pH above pH 9.5 (Jones and Handreck, 1967). Calcium oxalate phytoliths are even more susceptible to damage than silicon dioxide phytoliths. Also, fungi will form calcium oxalate crystals along their hyphae (both druses and raphide crystals) (Arnott & Webb, 1983) and therefore the calcium oxalate that is found may result from fungal growth rather than from plant calcification. Marshall (1992) reported finding calcium-containing crystals on Linum from the Franklin Expedition, determined through Energy Dispersive X-Ray Analysis (EDXA). Perhaps fungal hyphae played a role in that scenario; the sample was reportedly dirty and contaminated with soil (N. Kerr, personal communication, March 6, 1996).

Historically amongst archeologists there is a conception that abundant silica is thought to only be in certain families of plants such as gramineae (grasses), cyperaceae (sedges), and equisetum (horsetail). Continued research is proving that significant amounts can be traced to other families (Rovner, 1971; Sangster and Parry, 1981). More of the research carried out on families other than gramineae, cyperaceae, and equisetum is from Japan or Korea (in the data base files the abstracts are in English, but the articles are in Japanese or Korean). Another belief is that calcium oxalate is a ubiquitous material that cannot be used in plant taxonomy. One can perhaps understand an archaeologist's decision to dispense with consideration of calcium oxalate phytoliths. In paleoenvironments there may be no calcium oxalate left in the soils, since soils are naturally acidic and therefore calcium oxalate may survive only in a limited number of instances.

Perhaps the greatest limitation comes back to the lack of a comprehensive, collectively upheld classification system. The field is still new and therefore one can understand the lack of sufficient background material. Each new study provides additional keys for a specific series of plants. My research addressed the concern of classifying yet another series of plants for the specific purpose of identification of highly degraded cellulosic materials. To avoid being limited in scope, the broadest definition of phytolith was used, and included all inorganic substances that are inherently a part of a plant, and that may persist through degradation.

# CHAPTER 3: MATERIALS AND METHODS

## Materials

The plant materials used in the research were provided by the Philippine Fibre Development Authority (FIDA); were from the University of Alberta, Human Ecology Department, Bast Fibre Research Collection; or they were harvested and/or collected by myself.

The plant materials examined for the study were *Musa textilis* Née, or **abaca**, leaf sheath; *Musa textilis* Née leaf sheath fibre and fibre products; *Musa*, not *textilis*, leaf sheath fibre; *Agave cantala*, or **maguay**, leaf segment; *Agave* leaf fibre; *Agave* rope, from leaf fibre; *Boehmeria nivea* (L) Gaud., or **ramie,** rove, from stem fibre; *Corchorus capsularis* L., or **jute**, rove, from stem fibre; *Cannabis sativa* L. (variety Kompolti),or **hemp**, stem; *Cannabis sativa* L. "summer" cloth, from stem fibre; *Agave sisalana*, or **sisal**, stem fibre; and *Spinacia oleracea*, or **spinach**, leaf. The plant organs, or parts, were examined to establish reference data about the location of inorganic compounds, the leaf fibres and stem fibres were examined to determine if inorganic materials can be expected to be found on harvested fibres, and the textile products were examined to determine if inorganic materials can be expected to survive the manufacturing process. The *Musa*, species not *textilis*, was inadvertently sent by FIDA to contrast with the *Musa textilis* Née specimens, but the species was not designated. The Spinacia oleracea was examined in control trials for two of the experimental processes. Table 2 outlines the source of each material used in the study, the specimen description and condition, as well as the sample selection process, and the treatments applied for data collection. Each sample was assigned a number according to the scheme outlined on Table B1, Appendix B; for each sample there is a corresponding numbered data sheet.

The *Agave* leaf fibre and the *Agave* rope from leaf fibre are without determined species designations. The two specimens are from the University of Alberta, Human Ecology Department, Bast Fibre Research Collection; the courser leaf fibre rove is bound together by the *Agave* rope specimen. The pair were collected in Guatemala and are recorded as being *Agave cantala*, or **maguay** (the rove), and another bast fibre (the rope). The crystals present in the ash following thermal ashing of a set of rope samples indicated that it is an *Agave*, however, the determination of the species of *Agave* used for the rope, or that is in the rove, is not definite. *Agave cantala* is native to Mexico, whereas *Agave fourcroydes*, or **henequen**, and *Agave sisalana*, or **sisal** are found throughout Central America (Cook, 1984, pp. 27- 33). *A. fourcroydes* is cited by most authors as being the dominant agricultural crop in Central America (Gentry, 1982; Nobel, 1988; Nobel, 1994), with approximately 200,000 hectares (500,000 acres) of *A. fourcroydes* being cultivated in 1993 (Nobel, 1994, p. 40). Despite the dominance of *A. fourcroydes* and *A. sisalana*, other wild *Agave* stands in Central America yield fibres used in textiles, including *Agave lechugilla*, *Agave striata*, and *Agave salmiana* (Gentry, pp.17 - 18; Maiti, 1980, p. 170). *A. salmiana* is not likely the source of the fibre rove, as a result of the described fineness of the fibres taken from the conal bud of this species differing from the coarseness of the fibre rove, and because the rove came from a village on Lake Atitlan where the plants were cultivated for rope as a major industry for the village (N. Kerr, personal communication, February 21, 1996). The rove and the rope are likely either maguay or henequen.

**Table 2**. Details About the Materials Used in the Study.

| Plant Material Specimen | Specimen Source | Specimen Description | Specimen Condition | Sample Selection from Specimen | Sample Treatments (see Table 3, as well as the discussion of treatment methods in this chapter of the thesis) |
|---|---|---|---|---|---|
| ***Musa textilis* Née (*leaf sheath*)** | **Philippine Fibre Development Authority (FIDA)**<br>Specimens A - D from second shipment (August 1995), and Specimen E from first shipment (July 1995). | **Mean sheath dimensions:**<br>738 mm long<br>74.1 mm wide<br>0.655 mm deep (mean of 0.21mm near laminar edge and 1.1 mm near midrib)<br>Colour: tan to brown; darker and spotted near flared base; dark brown stripe, more noticeable on abaxial surface; adaxial surface is darker and shinier.<br>Texture: abaxial surface has more ribbing; adaxial surface has stomatal crypts. | Specimen A-D:<br>arrived dehydrated, flat and folded in half lengthwise, and appeared to be cleaned, perhaps washed.<br>Specimen E:<br>arrived dehydrated, curled and folded in half lengthwise, and appeared to be soiled – surface has a granular texture compared to specimens A - D. | Samples were taken from 22mm below leaf sheath specimen apex and from near the midrib and laminar edge.<br>Burn tests were carried out on 22 mm x 5 mm x 0.75 mm, longitudinal samples taken from near the apical midrib region.<br>Chemical digestion and crucible thermal ash samples consisted of 22 mm long transverse sections taken from 10cm below the apex. | ◆Mechanical sectioning, as well as burn and fibre twist tests,<br>◆Chemical Digestion,<br>◆Crucible Thermal Ashing at 500°C,<br>◆Light microscopy. |

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| ***Musa textilis* Née (*leaf sheath fibre* products)** | Philippine Fibre Development Authority (FIDA)<br>In addition to the *Musa* leaf sheath specimens, FIDA sent specimens of various grades of fibre (including WS, or wide strip grade, and G, or good grade) in a "Miniature Specimens of Philippine Fibres" brochure, as well as a roving of good grade fibre, various paper and pulp specimens, and specimens of "Abaca Fibrecraft". | Wide Strip (WS) Fibre:<br>Residual grade WS "fibres" retain epidermal (adaxial) tissue and adjoining fibre and fibrovascular bundles, as well as corticular tissue, including some aerenchyma. The colour and texture are similar to the *Musa* leaf sheath adaxial surface.<br>Good (G) Fibre:<br>G "fibres" consist of one to three fibre bundles, which may be fibrovascular, and some parenchymous tissue, but not any epidermal tissue. They are smooth, and lustrous; they are flexible, but not pliable.<br>Paper:<br>*Musa*, or Abaca paper consists of a mat of ultimate fibres with occasional fibre bundles, vascular tissue, epidermal tissue, lactiferous elements, and other debris. The colour is cream; the hand is soft.<br>Pulp:<br>*Musa*, or Abaca pulp is a denser version of *Musa* paper.<br>"Linen":<br>*Musa*, or Abaca "linen" is a balanced plain-weave cloth woven from elements consisting of more than one fibre bundle (see description of good grade fibre). The elements are crimped and have a rectangular perimeter as a result of weave structure (compression) forces. The colour is straw yellow; the textile will not drape. | Wide Strip (WS) Fibre:<br>Desiccated WS fibres are not coherent; they are brittle and inflexible, and contain the colour variations of the leaf sheath adaxial surface. The specimens received were clean and not extraneously damaged.<br>Good (G) Fibre, Paper, Pulp, and "Linen":<br>Desiccated, clean and not extraneously damaged. | Wide Strip (WS):<br>Mean dimensions of samples taken randomly from specimen:<br>22.0 mm long<br>1.46 mm wide (range 1.2 mm to 1.8 mm)<br>Good (G):<br>Mean dimensions of samples taken randomly from specimen:<br>22.0 mm long<br>0.36 mm wide (range: 0.2 mm to 0.6 mm)<br>Paper:<br>Dimensions of samples:<br>22 mm x 2 mm x 0.5 mm<br>Pulp:<br>Dimensions of samples:<br>3 mm x 3mm x 1 mm<br>"Linen":<br>Samples of 22 mm warp and filling fibres were selected for examination. | Wide Strip (WS):<br>♦Mechanical sections; burn and fibre twist tests,<br>♦Chemical Digestion,<br>♦Crucible Thermal Ashing at 500°C,<br>♦Glass Plate Ashing at 400°C, 500°C, 600°C,<br>♦Cold $O_2$ Plasma Ashing,<br>♦Light, electron and confocal microscopy.<br>Good (G):<br>♦Mechanical sections; burn test,<br>♦Chemical Digestion,<br>♦Crucible Thermal Ashing at 500°C<br>♦Glass Plate Ashing at 500°C<br>♦Cold $O_2$ Plasma Ashing<br>♦Light and electron microscopy.<br>Paper:<br>♦Mechanical sections; burn test,<br>♦Crucible Thermal Ashing at 500°C,<br>♦Glass Plate Ashing at 500°C,<br>♦Light microscopy.<br>Pulp:<br>♦Mechanical sections; burn test,<br>♦Crucible Thermal Ashing at 500°C,<br>♦Light microscopy.<br>"Linen":<br>as per paper. |

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| ***Musa*** (not *textilis, leaf sheath fibre*) | Philippine Fibre Development Authority (FIDA) | **Residual Grade Fibre:**<br>FIDA sent one specimen of non-*textilis Musa* fibre, similar to WS grade fibre (see description), but darker in value, to act as contrast to *Musa textilis* Née fibres. | Desiccated, clean and not extraneously damaged. | Samples of 22 mm long fibres were selected for examination. | ♦Mechanical sections,<br>♦Chemical Digestion,<br>♦Glass Plate Ashing at 500°C,<br>♦Light microscopy. |
| ***Agave cantala*** (*leaf* **segment)** | University of Alberta, Edmonton, Grounds and Maintenance<br>Leaf segment specimen from *Agave cantala*, or Century Plant in place in the concourse between the International Student Mall and Rutherford Library | **Segment of leaf** specimen is from the laminar edge near the leaf apex.<br>**Specimen Dimensions:**<br>140 mm long<br>35 mm wide<br>(depth varies due to ribbing)<br>Specimen taken from the laminar edge, near the apex; one spine is in place.<br>**Colour:** tan, to orange, to brown on epidermal surfaces; the spine is dark brown.<br>**Texture:** both the abaxial and adaxial surfaces are ridged; the leaves of the plant are patterned on the abaxial surface by the leaves they once concealed, while on the adaxial surfaces they are patterned by the leaves that once concealed them; the surface is matte; the spines of the plant are shaped like rose thorns and they are smooth, shiny, hard and sharp. | The specimen is desiccated and scarred, as well as folded and edge abraded.<br>The one spine is broken at the tip.<br>Some fibres are exposed where the leaf has been torn. | Samples of 17 mm x 7 mm segments from near the laminar edge were selected for examination. | ♦Mechanical sectioning, as well as burn and fibre twist test. |
| *Agave* (*leaf fibre*) (continued next page.) | Guatemala<br>Course *Agave* rove of "fibres" collected by Dr. | **Course Fibre** specimen composed of decorticated, but not completely stripped, fibrous material. The "fibres" retain epidermal tissue and adjoining fibre and fibrovascular bundles, as well as corticular tissue. Individual fibre bundles | Desiccated, clean and not extraneously damaged. | A sample was taken from the middle of the roving, and then samples consisting of one to three fibre bundles were | ♦Mechanical sections; burn and fibre twist tests,<br>♦Chemical Digestion,<br>♦Glass Plate Ashing at 500°C,<br>♦Cold $O_2$ Plasma Ashing. |

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| ***Agave***<br>**(*leaf fibre*)**<br>(continued) | Nancy Kerr,<br>University of<br>Alberta, Edmonton, | separate readily when specimen is handled<br>Colour: straw yellow and shiny with some pale green material (chlorencymous) on wide strips.<br>Texture: smooth and shiny (fibre) - matte (parenchyma) surface with loose fibres. Ridges of fibre bundles visible on wider strips. | | pulled randomly from the larger sample.<br>Mean dimensions of samples:<br>22.0 mm long<br>0.50 mm wide (range: 0.2 mm to 0.75 mm). | ♦Light, and electron microscopy. |
| ***Agave***<br>**(rope,**<br>**from *leaf***<br>***fibre*)** | Guatemala<br>Course *Agave* fibre, tied around the course fibre bundle collected by Dr. Nancy Kerr, University of Alberta, Edmonton, | Rope is composed of "z" spun fibre bundles.<br>Colour: straw yellow and shiny.<br>Texture: smooth and shiny with extraneous matte parenchyma. | Desiccated, clean and not extraneously damaged.<br>Fibre bundles exhibit a twisting form due to the spinning process during rope formation. | A sample was taken from one end of the rope, and then samples consisting of one to three fibre bundles were pulled from the larger sample.<br>Mean dimensions of fibre bundles in samples:<br>22.0 mm long<br>0.20 mm wide | ♦Mechanical sections; burn and fibre twist tests,<br>♦Glass Plate Ashing at 500°C,<br>♦Light, and electron microscopy. |
| ***Boehmeria nivea*** **(L) Gaud.**<br>**(rove, from *stem fibre*)** | University of Alberta,<br>Human Ecology Department, Bast Fibre Research Collection. | Rove of fibre ultimates.<br>Colour: white and shiny.<br>Texture: fine, soft, smooth and pliable. | Desiccated, clean and not extraneously damaged. | A sample was taken from the middle of the rove, and then samples consisting of ten fibre ultimates were pulled from the larger sample.<br>Mean width of collapsed fibres:<br>52.63 μm (range: 40.0 μm to 75.0 μm). | ♦Mechanical sections; burn and fibre twist tests,<br>♦Chemical Digestion,<br>♦Glass Plate Ashing at 500°C,<br>♦Cold $O_2$ Plasma Ashing,<br>♦Light, and electron microscopy. |

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| *Corchorus capsularis* L. (rove, from *stem fibre*) | **University of Alberta,** Human Ecology Department, Bast Fibre Research Collection. | **Rove of fibre bundles** composed of 2-3 bundles, with varied amounts of adhering parenchymous tissue; occasional wood fibre segments<br>**Colour:** straw yellow to amber colour and a matte to shiny appearance.<br>**Texture:** uneven texture, and not pliable. | Desiccated, clean and not extraneously damaged. | A sample was taken from the middle of the rove, and then samples consisting of fiber bundles were taken from the larger sample.<br>Mean diameter of fibre bundles:<br>76.67 µm (range: 35.0 µm to 155.0 µm). | ♦ Mechanical sections; burn and fibre twist tests,<br>♦ Chemical Digestion,<br>♦ Glass Plate Ashing at 500°C,<br>♦ Cold $O_2$ Plasma Ashing,<br>♦ Light, and electron microscopy. |
| *Cannabis sativa* L. (variety *Kompolti*) (*stem*) | **University of Alberta,** N. Kerr, Human Ecology Department, Bast Fibre Research Collection. | **Specimen Dimensions:** stem length 750mm, apical end 50mm circumference, base end 130mm circumference, internodal regions range 100 - 200mm<br>**Colour:** Stem epidermis and attached leaves are matte green. Secondary xylem tissue that is visible at break points is straw yellow colour and the pith is silvery-white.<br>**Texture:** Stem epidermis has a nap, most noticeable in the apex to base direction; the leaves are brittle. | Desiccated, and clean. Stem broken and torn at points where folded during packing for delivery. Debris in bottom of packaging material consists of leaf fragments. | Transverse and radial sections taken for examination near apex and base of specimen.<br>Burn characteristics were observed on samples measuring<br>15mm x 2mm x 1.5mm. | ♦ Mechanical sections; burn and fibre twist tests,<br>♦ Light, and electron microscopy. |
| *Cannabis sativa* L. ("summer cloth", from *stem fibre*) | **University of Alberta,** Purchased from Hemp Textiles International, 3200-30th Street, Bellingham, WA, USA 98225 | **Specimen Dimensions:** Warp and filling yarns unraveled from 22mm x 22mm specimen taken from a larger meterage specimen.<br>**Colour:** textile specimen is straw yellow in colour, and has a sheen.<br>**Texture:** the yarns are crimped due to the compression forces of the plain weave structure. | Clean and not extraneously damaged. Yarn is crimped due to compression forces of weave structure. | Fibres taken from yarn samples drawn from bundle of warp and filling yarns unraveled from specimen. | ♦ Mechanical sections; burn and fibre twist tests,<br>♦ Chemical Digestion,<br>♦ Glass Plate Ashing at 500°C,<br>♦ Cold $O_2$ Plasma Ashing,<br>♦ Light and electron microscopy. |

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| *Agave sisalana* (*stem fibre*) | University of Alberta,<br>Human Ecology Dept., Bast Fibre Research Collection. | **Specimen Dimensions:** 22mm segment of rope taken from larger rope specimen.<br>**Colour:** dyed pink and yellow fibre, with a sheen.<br>**Texture:** fibres are "z" twisted due to the plying of the yarn sample, otherwise they are smooth. | Dyed, desiccated, clean and not extraneously damaged. | Fibre bundles taken from the rope specimen. | ♦Glass Plate Ashing at 500°C<br>♦Light microscopy |
| Spinacia oleracea (*leaf*) | Researcher grown and harvested. | **Specimen:** each leaf taken fresh to the lab in deionized water, and kept moistened for processing.<br>**Colour:** green. | Fresh specimens, clean and not extraneously damaged. | Chemical digestion and $O_2$ plasma ashing samples consisted of 22 mm squares of fresh leaf. | ♦Chemical Digestion.<br>♦Cold $O_2$ Plasma Ashing.<br>♦Light microscopy. |

## Methods Of Data Collection

The methods used in the study included mechanical sectioning, clearing, chemical digestion, thermal ashing, oxygen plasma ashing, light microscopy, polarized light microscopy, scanning electron microscopy, energy dispersive x-ray analysis, confocal microscopy, and critical point drying for scanning electron microscopy. Table 3, indicates which methods were used with each plant specimen.

### Mechanical Processing (Method A)

Specimens and samples were mechanically processed according to various sources (AATCC Test Method 20, 1994; Berlyn & Miksche, 1976; Catling & Grayson, 1982; Ishii & Takiyama, 1989). For each specimen or sample, mechanical processing was guided by a mechanical processing checklist, and each step of the process was recorded on a mechanical processing record table (see Table B2 and Table B3, in Appendix B). Mechanical processing began with a gross description, a condition notation, and a context explanation. Next, radial and/or tangential (longitudinal) and transverse (cross) sections were prepared, mounted, and recorded following a set of observation criteria. In two instances, *Musa* and *Agave*, samples were cleared (see p. 55) to assist with viewing, and in preparation for confocal microscopy. In one instance, a set of *Musa* samples were critical point dried (see p. 54), in preparation for scanning electron microscopy. To complete the mechanical processing, polarized light response, twist test, and burn characteristics were noted. All of the data collected were recorded in either the laboratory book or on data sheets, which were cross-referenced (see Figure B1 in Appendix B).

The gross description of each sample or specimen included dimension, colour, and texture, as well as, for textile specimens, weave structure, yarn ply, and ply twist. The

**Table 3.** Phytolith isolation methods, and number of specimens used with each method.

| **METHOD**<br>* quantitative & qualitative yield<br>* availability of materials & equip | *safety<br>* time<br>* cost | **A Mechan-ical** | **B Chem-ical** | **C Dry Ashing Crucible** | **D Dry Ashing Glass Plate 400°C** | **E Dry Ashing Glass Plate 500°C** | **F Dry Ashing Glass Plate 600°C** | **G Cold Plasma Ashing** |
|---|---|---|---|---|---|---|---|---|
| **MATERIALS**<br>* sample weight<br>* sample size relative to plant<br>* qualitative description<br>- collection context<br>- location on plant<br>- context of plant anatomy & metabolism<br>- processing methods<br>- drawings of plant<br>- micrograph as possible<br>- kept sample | ***Musa***<br>(leaf sheath) | **0001a** | **0005a** | **0010a** | | | | |
| | | b | b | 0011a | | | | |
| | | c | c | 0012a | | | | |
| | | d | d | 0013a | | | | |
| | | e | e | | | | | |
| | ***Musa***<br>(W5) | **100a** | **pretrials** | **124** | **108a** | **113a** | **109a** | **116a** |
| | | b | (102) | 125 | b | b | b | b |
| | | c | (106) | 126 | c | c | c | c |
| | | d | (107) | 127 | d | d | d | d |
| | | | | 128 | | | | e |
| | ***Musa***<br>(G) | **230a** | **242a** | **210** | | **200** | | **206a** |
| | | b | b | 211 | | 201 | | b |
| | | c | c | 212 | | 202 | | c |
| | | d | d | 213 | | 203 | | d |
| | | e | e | 214 | | 204 | | e |
| | ***Musa***<br>(paper) | **250a** | | **260** | | **265a** | | |
| | | b | | 261 | | b | | |
| | | c | | 262 | | c | | |
| | | d | | 263 | | d | | |
| | | e | | | | e | | |
| | ***Musa***<br>(pulp) | **270a** | | **276** | | | | |
| | | b | | 277 | | | | |
| | | c | | 278 | | | | |
| | | | | 279 | | | | |
| | | | | | | | | |
| | ***Musa***<br>("linen") | **300W/F** | | **310** | | **320a** | | |
| | | Wb/Fb | | 311 | | b | | |
| | | Wc/Fc | | 312 | | c | | |
| | | Wd/Fd | | 313 | | d | | |
| | | We/Fe | | | | | | |
| | ***Musa***<br>(not t.) | **1100a** | **1101a** | **1102** | | **1103a** | | |
| | | b | b | | | b | | |
| | | c | c | | | c | | |
| | | d | d | | | d | | |
| | | e | | | | e | | |
| | ***Agav.***<br>(leaf) | **350** | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | ***Agav.***<br>(course fibre) | **400a** | **410 a** | | | **420a** | **1050** | **426a** |
| | | b | b | | | b | | b |
| | | c | c | | | c | | c |
| | | d | d | | | d | | d |
| | | e | e | | | e | | e |
| | ***Agav.***<br>(rope) | **450a** | | | | **465a** | | |
| | | b | | | | b | | |
| | | c | | | | c | | |
| | | d | | | | d | | |
| | | e | | | | e | | |

| **METHOD**<br>* quantitative & qualitative yield<br>* availability of materials & equip. | *safety<br>* time<br>* cost | **A Mechanical** | **B Chemical** | **C Dry Ashing Crucible** | **D Dry Ashing Glass Plate 400°C** | **E Dry Ashing Glass Plate 500°C** | **F Dry Ashing Glass Plate 600°C** | **G Cold Plasma Ashing** |
|---|---|---|---|---|---|---|---|---|
| **MATERIALS**<br>* sample weight<br>* sample size relative to plant<br>* qualitative description<br>- collection context<br>- location on plant<br>- context of plant anatomy & metabolism<br>- processing methods<br>- drawings of plant<br>- micrograph as possible<br>- kept sample | ***Boeh***<br>(roving) | **500a** | **510** | | | **520a** | | **526a** |
| | | b | 511 | | | b | | b |
| | | c | 512 | | | c | | c |
| | | d | 513 | | | d | | d |
| | | e | 514 | | | e | | e |
| | ***Corc.***<br>(fibre) | **600a** | **610a** | | | **620a** | | **626a** |
| | | b | b | | | b | | b |
| | | c | c | | | c | | c |
| | | d | d | | | d | | d |
| | | e | e | | | e | | e |
| | ***Cann***<br>(stems) | **700a** | | | | **720a** | | |
| | | b | | | | b | | |
| | | c | | | | c | | |
| | | d | | | | d | | |
| | | e | | | | e | | |
| | ***Cann***<br>summer cloth | **800W/F** | **810** | | | **820a** | | **826a** |
| | | Wb/Fb | 811 | | | b | | b |
| | | Wc/Fc | 812 | | | c | | c |
| | | Wd/Fd | 813 | | | d | | d |
| | | We/Fe | 814 | | | e | | e |
| | ***Agav.***<br>(sisal.) | **1250** | | | | **1270a** | | |
| | | | | | | b | | |
| | | | | | | c | | |
| | | | | | | d | | |
| | | | | | | e | | |
| | ***Spin.***<br>(leaf) | **1300** | 1310 | | | | | 1326 |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

*Agav.* = *Agave cantala*, or **Maguey; also *Agave sisalana***
*Boeh.* = *Boehmeria nivea* (L.) Gaud, or **Ramie;**
*Cann.* = *Cannabis sativa* L., or **Hemp**;
*Corc.* = *Corchorus capsularis* L., or **Jute;**
*Musa* = *Musa textilis* Née, or **Abaca**, or **Manila Hemp.**
***Spin.* = *Spinacia oleracea*, or spinach.**
**W/F = Warp/Filling of woven fabrics**

Drying Twist test (Marshall, 1992; Schaffer, 1981a) was applied to fibres from each specimen. Establishing context for each specimen included collection context, the context of the specimen within plant anatomy and metabolism, and the processing methods used to produce each specimen.

Criteria used when viewing longitudinal sections included: mountant, magnification, dimensions, tissue types, cell types, fibre cell end, fibre cell markings, fibre cell wall, fibre cell lumen, crystals, and debris other than cellular. Transverse section viewing criteria included: mountant, magnification, dimensions, tissue types, cell types, fibre cell wall, fibre cell lumen, cell-cell interactions, cell-cell spatial relationships, crystals, and debris other than cellular. Burn characterization included a description of the specimen's behavior: near flame, at ignition, in flame, flame colour, ash colour, and ash texture.

Specimen dimensions were determined microscopically using a calibrated eyepiece micrometer. Sectioning of stem or leaf sheath samples was done manually using a mounted and weighted steel blade. The best sections resulted when specimens were rehydrated for 24 hours prior to sectioning. Sectioning of smaller samples was done using a hand-held microtome or a perforated plastic plate according to AATCC Test Method 20, section 9.3 (1994). To avoid contamination of samples by glass shards, the glass plates and glass cover slips were cleaned with Sparkleen® in warm tap water, then rinsed in deionized water and placed in a dust-free area to dry. A control slide of glass plate and glass cover slip shards was prepared and continually referenced until immediate recognition of shards was possible. Mountants included air, deionized water, or glycerol. In most cases the samples were viewed sequentially in air, then deionized water, then glycerol[2].

Sections were placed on a glass plate and observed in air. Deionized water was added to the sample and observed. A glass cover slip was placed over the sample and the glass plate placed on an inverted halogen lamp for heating. This "boiling" process forced the air from the sample[3]. As the sample was boiling, a glass plate with a drop of glycerol was being warmed above the microscope lamp. Occasionally, additional deionized water was added to the sample under the glass cover slip. Once the boiling was complete, the sample was immediately transferred to the warmed glycerol and allowed to cool. Mounted samples were made semi-permanent[4] by bordering their glass cover slip with a clear nail polish seal.

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[2] Calcium oxalate crystals were discernable in deionized water and clearly viewed in glycerol; silica was discernable in deionized water but not easily viewed in glycerol, as a result of similarity of refractive indicies.

### Chemical Digestion (Method B)

Sets of samples were chemically ashed according to Jones (1988), with modifications to accommodate the digestion of fibrous materials. Samples weighing 0.5 grams were rehydrated at least 24 hrs. in deionized water, dried of excess moisture, and placed in 400 ml glass beakers in a fume hood. The samples were covered with 10 ml of 30% hydrogen peroxide ($H_2O_2$) and allowed to sit for thirty minutes. The potassium dichromate ($K_2Cr_2O_7$, approximately 0.25 grams) was then added. (I wore gloves, and had the fume hood closed below six inches and the fume hood fan turned on.) Within less than one minute a violent exothermic reaction began and ended, and the solution changed from dark purple to ochre or yellow-brown, depending on the material being treated. The reaction was then recommenced by adding, with a pipette, successive 1.0 ml portions of 30% hydrogen peroxide, washed down the side of the beaker to move adhering fibres back into the solution. A control sample of Spinacia oleracea was digested in one minute and 20 seconds, however, all the fibrous samples required repeated applications of 30% hydrogen peroxide. The reaction was maintained for a maximum of one hour in each case. The most thorough digestion was achieved when the reaction was maintained for five cycles, and, then, twice, let to sit overnight, and put through five additional cycles.

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[3] This technique was developed following Catling and Grayson's (1982, p. 6) method of boiling fibre samples to remove air prior to mounting.

[4] After eight months the nail polish and glycerol continue to endure .

Satisfactorily digested sample solutions were transferred to 150 ml beakers, covered, to avoid evaporation, and set aside for prolonged sedimentation to occur. The 400 ml beakers were repeatedly rinsed with deionized water to ensure thorough transfer of any crystals. At twelve hour intervals the clear supernatant solution was removed using a pipette, avoiding disturbances, and the remaining solution, with crystals, was transferred to smaller capacity beakers and test tubes, the final transfer being to a 3 ml test tube. At each transfer, a portion of the removed supernatant was viewed under the microscope. Each transfer was followed by dilution with deionized water and prolonged sedimentation. The 3 ml test tubes were inverted on glass plates for prolonged sedimentation to deposit the crystals on the slide. In most cases, the entire contents of the 3 ml test tube were examined. Mounting and examination were the same as for mechanical sectioning.

### Thermal Ashing, Crucible (Method C)

Sets of four or five *Musa* samples were thermal ashed according to AATCC Test Method 78 (1993), but the muffle furnace temperature was modified to 500°C to limit the distortions of the inorganic compounds that may have been left in place (see discussion in *Results* section of the thesis), and weighing paper was used in place of weighing bottles. Following charring over a gas burner, the crucibles were placed in the muffle furnace, uncovered, and thermal ashed for 20 minutes. The oven was then turned off and the crucibles were allowed to cool until the muffle furnace temperature had dropped to approximately 100°C before being placed in desiccators and weighed to a constant weight (using a 105°C drying oven, and a one hour wait time between weighings).

The data from the thermal ashing in crucibles method were used to quantitatively and qualitatively compare the ash content of seven forms of *Musa* specimen: *Musa* leaf sheath, *Musa* wide strip grade (WS) fibre bundles, *Musa* good grade (G) fibre bundles, *Musa* paper, *Musa* pulp, *Musa* "linen", and *Musa* (not *textilis*).

### Thermal Ashing, Glass Plate (Methods D, E, and F)

Samples were thermal ashed between glass plates according to Lanning, Ponnaiya, & Crumpton (1958), however, the samples were viewed successively in air, deionized water, and glycerol, rather than in Canada balsam. In the pretrials with *Musa textilis*, when the methods were being tested, samples were ashed at three different temperatures in the muffle furnace: 400°C (method D), 500°C (method E), and 600°C (method F). As a consequence of the results of the pretrials, 500°C, was chosen for the balance of the sample treatments. At 400°C the ashing was incomplete, and, therefore, viewing was inhibited, and at 600°C the silica bodies and calcium oxalate crystals were distorted, and the glass plate and cover slip warped.

Glass plates and glass cover slips were cleaned with Sparkleen® in warm tap water, then rinsed in deionized water and placed in a dust-free area to dry prior to use. Sets of five slides, with five fibres or fibre bundles each, were placed on a tile and put into the muffle furnace preset to 500°C. Achieving 500°C from 20°C took about 25 minutes. After 20 minutes at 500°C, the oven was then turned off and the slides were allowed to cool until the muffle furnace temperature had dropped to approximately 100°C.

The three sets of spodograms (slides of ash residue) from the pretrials were evaluated relative to one another. Subsequently, sets of spodograms from 500°C ashings were examined according to the following scheme: all five slides viewed in air, two slides viewed in deionized water, two slides viewed in glycerol.

### Oxygen Plasma Ashing (Method G)

Samples were ashed in an oxygen plasma at a low temperature (maximum 25.3°C) after a method described by Hozumi, Hutoh, & Umemoto (1972). Figure 2 is a schematic of the apparatus[5] that was used to treat the samples with oxygen plasma. Samples were placed in a sealed quartz tube with oxygen flowing in at a constant rate

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[5] The apparatus was configured by Stuart Schroeder, Analytical Chemistry PhD student, University of Alberta, Department of Chemistry.

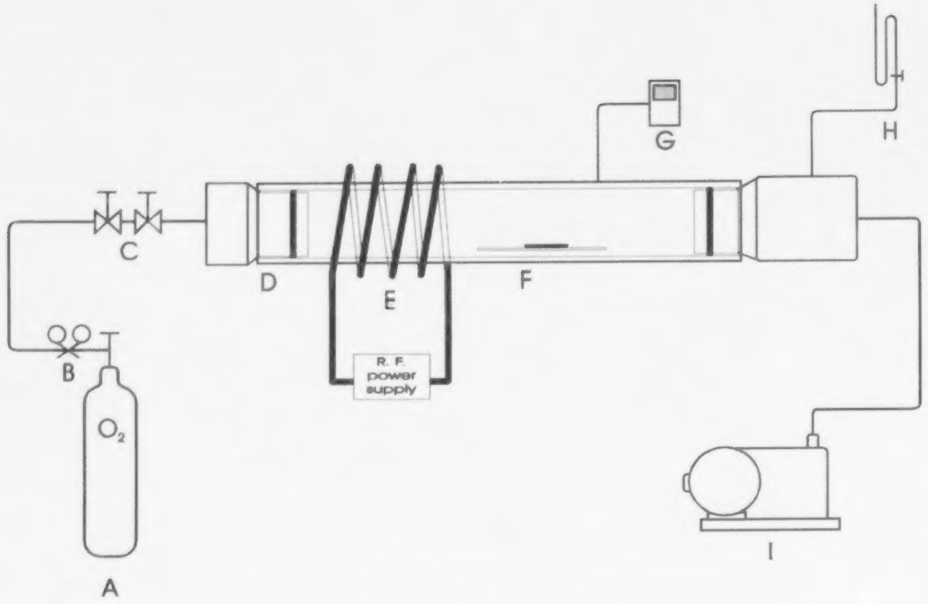

**Figure 2.** Schematic diagram of low-temperature oxygen plasma ashing apparatus[6]: (A) oxygen tank; (B) oxygen tank pressure gauge; (C) needle valve, Whitey flow meter; (D) quartz tube apparatus, including 22mm x 200mm quartz tube, brass inlet couple, and brass outlet couple; (E) high frequency, water-cooled coil with power supply, ICP2500, Plasma-Therm Inc., and RF generator, type HPF 2500F; (F) glass plate with sample in place; (G) thermocouple; (H) Wallace & Tiernan FA160 absolute pressure gauge; (I) Edwards 2-stage vacuum pump, 3/4 H.P.

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[6] The apparatus was configured by Stuart Schroeder, Analytical Chemistry Ph.D. candidate, University of Alberta, Department of Chemistry.

of 14 cc $min^{-1}$ and a pressure maintained at 0.5 Torr. A radio frequency power supply of 27mHz was maintained at 30 watts (50 incident watts, less 20 reflected watts) to create a gentle plasma, that would remove the organic matter at a slow rate, to avoid disturbances. Despite the gentle nature of the process, some disruption did occur during ashing, and, in pretrials, electrostatic forces caused further disruptions when the ashed sample was being removed from the quartz tube. The order was also disrupted during storage and handling of the slides because the method did not include mounting the ashed samples in a permanent mountant. The final plasma ashing experiments yielded useful qualitative results.

Umemoto, Hutoh, & Hozumi (1973) reported ashing times of 2 - 3 hours for most leaf tissues, and 5 - 6 hours for leaf costa. Five hours was not sufficient to completely ablate the larger fibre bundles. After 18 hours, one sample of *Musa textilis* (a wide strip grade fibre, approximately 1.2 mm in diameter) was bleached, but digested only to a minimal degree. A control sample of Spinacia oleracea was ashed in 5 hours and displayed an expected array of calcium oxalate druse crystals. Smaller bundles (approximately 0.3mm - 0.6mm diameter) of *Musa* fibres and *Agave* fibres were sufficiently ashed in 6 - 7 hours. *Boehmeria* ultimates and small *Corchorus* fibre bundles were completely ashed in less than 7 hours.

### Light Microscopy

Sections were examined using a Kyowa FK916-003, Unilux-11 binocular microscope with a 10x eyepiece and, 4x, 10x, 40x, and 100x (oil immersion) objective lenses. Samples that were mounted in glycerol could be rotated, which allowed a three dimensional reconstruction to be drawn. In combination, longitudinal and transverse sections viewed with the light microscope, and longitudinal sections viewed stereoscopically, allowed for reconstruction of the location of crystals within the plant specimens. Polarized light microscopy was used to view the calcium oxalate crystals.

Photomicrographs[7] were prepared on a Carl Zeiss photomicroscope, with a Winder M 35 camera attachment, using brightfield and differential interference (DIF, or polarized light) contrast.

### Scanning Electron Microscopy (SEM)[8]

Samples were mounted on stubs and sputter coated with gold in preparation for scanning electron microscopy (SEM). One sample of *Musa* was critical point dried according to Chen (1994). Critical point drying is used to retain the morphology of specimens by maintaining equivalent internal and external pressures with carbon dioxide ($CO_2$) gas during the drying process. Samples in 100% ethanol are inserted into a chamber which is flooded with liquid $CO_2$. The chamber is heated under pressure to form $CO_2$ gas and then it is evacuated.

Sets of stubs were mounted on a carrying stage and entered into the vacuum chamber of a Cambridge Stereoscan 250 electron microscope. The images were viewed using between 5KV and 15KV and working distances of between 10 mm and 20 mm. The images were recorded on black and white film, and as digital images on a compact disc.

### Energy Dispersive X-ray Analysis (EDXA)

A selection of crystals, cystolith bodies, and undetermined materials were examined for elemental content during the SEM process using energy dispersive x-ray analysis (EDXA). The readouts from the EDXA process provided data to support the likelihood that silica and calcium oxalate compounds were being viewed. For x-ray analysis of certain samples the KV on the SEM scope was set at 20.

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[7] The photomicrographs were taken by Dr. David Cass, Professor, University of Alberta, Department of Biological Sciences.

[8] The assistance provided by George Braybrook, Chief SEM Technician, University of Alberta, Department of Earth Sciences is gratefully acknowledged.

### Confocal Laser Scanning Microscopy (CLSM)[9]

The advantages of using confocal laser scanning microscopy (CLSM) include: elimination of out of focus glare, improved lateral resolution, the ability to do vertical as well as horizontal scans, the ability to create three dimensional reconstructions from compositions of vertical scans, and the ability to enhance and analyze images (Bhatnagar, 1995). Samples examined with CLSM need to exhibit autofluorescence, or be stained with fluorochromes that will fluoresce, with the wavelength of the laser light being used. Sections, to a maximum thickness of 300 μm can be viewed with CLSM (thicker sections need to be made transparent by clearing). The University of Alberta biological electron microscopy lab has a Multiprobe 2001 Confocal Laser Scanning Microscope, equipped with an argon/crypton laser with primary emission lines at 488 nm, 568 nm, and 647 nm.

Six samples sets were examined using the 488DF10 laser wavelength filter, with the phase contrast and polarizing capabilities of CLSM: *Musa* transverse section, *Musa* longitudinal section, *Musa* silica body, *Agave* longitudinal section, and *Agave* styloid and raphide crystals. An *Agave* styloid crystal was vertically scanned at 0.8 μm intervals, with only the polarizing filter in place, and a three dimensional reconstruction was produced.

In preparation for CLSM, the *Musa* and *Agave* samples were cleared according to Berlyn and Miksche (1976). Specimens, having been rehydrated in deionized water, were placed in a 5% solution of sodium hydroxide (NaOH). During several days of alkali treatment, the NaOH was replaced until it was clear. The specimens were then rinsed in three changes (five minutes each) of deionized water, and then immersed for 24 hours in a chloral hydrate (2,2,2-trichloro-1,1-ethanediol) solution (250 gr chloral hydrate: 100 ml deionized water). The specimens were then passed through a dilution sequence to pure deionized water, and, then, through a graded alcohol series to 95% ethanol.

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[9] The assistance provided by Rakesh Bhatnagar, Assistant Director, EM Lab, University of Alberta, Department of Biological Sciences is gratefully acknowledged.

# CHAPTER 4: RESULTS

## *Introduction*

Results were obtained from the study of seven phytolith distillation methods. The phytolith content of *Musa textilis* Née wide strip, or WS grade fibre was determined in the process of experimenting with the seven different methods. Subsequently, phytolith data were collected from six additional materials, using a maximum of four of the tested methods with each material. The comparison of the methods, and the phytolith data results, are both presented and discussed in this chapter of the thesis.

## Comparison Of Phytolith Distillation Methods

The seven methods which were tested and evaluated included method A, mechanical processing; method B, chemical digestion; method C, thermal ashing in a crucible; method D, glass plate thermal ashing at 400°C; method E, glass plate thermal ashing at 500°C; method F, glass plate thermal ashing at 600°C; and method G, ashing in an oxygen plasma. The evaluation criteria included safety, cost, time, accessibility, and yield. The cost criterion was divided into set-up costs and cost to process one sample. The time criterion was divided into time to complete one cycle and ten cycles, and the ability to process samples concurrently. The accessibility criterion included access to equipment and the ease of equipment operation. The post-treatment yield criterion was divided into three sub-criteria including the retention of phytolith positioning within the plant sample, the retention of phytolith morphology, and the ability to yield qualitative and/or quantitative data.

Table 4 summarizes the criteria for evaluating the seven phytolith distillation methods. The safety criterion indicated low risk for all but one of the methods (chemical digestion). The safety concerns of the chemical digestion method can be managed by using the precautions outlined in the methods section of the thesis. In general, all the methods were chosen, in part, due to their low risk health and safety factors. The evaluation of the methods' costs indicates that the highest set-up expense

**Table 4.** Summary of the criteria evaluating the methods tested in the study (see the thesis text, Table 5, and Table 6 for further explanation).

| Method | Safety | Cost[10] | | Time | | Accessibility | | Yield | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | set-up | per sample (after set-up) | time to complete one cycle: ten cycles | concurrent processing of samples | access to equipment | equipment operation | retention of phytolith positioning within plant | retention of phytolith morphology | qualitative or quantitative data |
| A) Mechanical Processing | Low risk[11] | 4,896.50 | 1.90 | Depends on sample, mean= $60_{min.}$:$600_{min}$ | No (each sample treated individually) | readily accessible | 1 skill required | very good, though some displacement in transverse and longitudinal section | good | qualitative data |
| B) Chemical Digestion (Method B) | Violent Chem. Reaction; $K_2Cr_2O_7$ Carcinogenic[12] | 10,517.00 | 4.56 | Over three days, $300_{min}$: $500_{min}$ (Including microscopy) | Yes (6 samples at once is manageable) | accessible | 2 skill required | lost, due to the digestion of the organic materials | good, though not possible to say for silica bodies | qualitative data |
| C) Thermal Ashing, Crucible | Low risk | 11,317.50 | 1.88 | Over five days, $360_{min}$: $720_{min}$ (Including microscopy) | Yes (5 samples at once is manageable) | accessible | 3 skill required | poor, due to the the need to transfer the ash to a slide after ashing | poor, due to preashing temperatures over gas burner | quantitative data (some qualitative data) |
| D) Thermal Ashing, Glass Plate, 400°C | Low risk | 6,422.50 | 1.50 | $240_{min}$: $480_{min}$ (Including microscopy) | Yes (5 samples at once is manageable) | accessible | 2 skill required | good, though some movement during ashing, must disrupt to view | fair, due to low degree of ashing, phytoliths can be difficult to discern | some qualitative data |
| E) Thermal Ashing, Glass Plate, 500°C | Low risk | 6,422.50 | 1.50 | $240_{min}$: $480_{min}$ (Including microscopy) | Yes (5 samples at once is manageable) | accessible | 2 skill required | good, though some movement during ashing; must disrupt to view | good-fair, some phytoliths retain good form, while some are distorted | qualitative data |
| F) Thermal Ashing, Glass Plate, 600°C | Low risk | 6,422.50 | 1.50 | $240_{min}$: $480_{min}$ (Including microscopy) | Yes (5 samples at once is manageable) | accessible | 2 skill required | fair-good, due to movement during ashing, must disrupt to view | poor, most phytoliths are distorted (degraded, or tarred and bent) | most qualities lost |
| G) Oxygen Plasma Ashing | Low risk | 29,265.50 | 1.49 | $480_{min}$: $4800_{min}$ [illegible] | No (each sample treated individually) | special arrangements | 1 skill required | good, though some movement during ashing | good | qualitative data |

**Note.**

| most desirable 1 | 2 | 3 | 4 | least desirable 5 |
|---|---|---|---|---|

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[10] See Table C1in Appendix C for full details.

[11] Low risk is in effect if operator is properly trained in the practical and theoretical use of all equipment and materials.

[12] Carcinogen IARC 23,205 (1980) Suppl. 7, 165 (1987) (BDH Inc., Material Safety Data Sheet, 1993).

is for the oxygen plasma ashing method, and the lowest set-up cost is for the mechanical processing method. In contrast, the per sample cost is lowest for the oxygen plasma ashing method, and mid-range for the mechanical processing method. The highest per sample cost is for the chemical digestion method.

If the "time to complete one sample and ten samples" is considered, oxygen plasma ashing is the least desirable method, and glass plate thermal ashing is the most desirable method. If only one sample is to be tested, the most desirable method, timewise, is mechanical processing. Even though oxygen plasma ashing required a great number of processing hours (between 7 and 18), the time need not be solely occupied by the plasma ashing process. At intervals (every 15 minutes in the exploratory trials, and every half to one hour in the experimental trials), the samples needed to be observed, and the time, power supply, pressure, and temperature needed to be recorded. In the current research, the balance of the time was used to complete some of the mechanical processing trials. Some of the methods allowed for concurrent processing of multiple samples, however, each sample eventually needed to be examined individually under the microscope (see Table 4). The ability to process samples concurrently affects the time to complete ten cycles, and, as a result, the ranking of the methods in time is affected.

The two accessibility criteria address the ease of obtaining and operating equipment. Due to the relative ease of obtaining a microscope and related supplies, *and* the relative ease of microscope operation, the mechanical processing method is the most desirable choice. If only the ease of obtaining equipment criterion is considered, then the oxygen plasma ashing method is considered the least desirable due to the lack of equipment availability (for the study an oxygen plasma apparatus was specifically built). Once built, the oxygen plasma ashing equipment is relatively easy to operate, which makes the method one of the more desirable ones. With regards to equipment operation, if one is attempting to quantify the phytolith yields, the thermal ashing in a crucible method requires a relatively high degree of skill to achieve constant weights (to 1/1000 gram, on an analytical balance), and, therefore, is relatively less desirable.

The final, and perhaps the most significant criteria evaluated, were the three phytolith yield criteria. No one method alone provided a clear indication of the positioning of the phytoliths within the plant sample or the retention of the phytolith morphology, and none of the methods provided both good quantitative and qualitative data. The yield results indicated that more then one method for distilling phytoliths should be used for any one specimen. Mechanical processing was useful for qualitatively describing the sample, and for viewing the morphology of the phytolith, however, the displacement of the phytoliths during sectioning made this method less desirable. With chemical digestion the relationship of the phytoliths to the plant sample was lost, however, clear examples of phytoliths were obtained in most cases. Quantifications of the chemically digested samples were not made. The samples that were thermal ashed in crucibles yielded some quantitative data, but the high temperatures during pre-ashing over a gas flame managed to distort the phytolith morphology, and the process of moving the ash from the crucible to the glass plate for microscope viewing tended to disorient the phytoliths. As a result, the appearance and orientation of phytoliths obtained from thermal ashing in a crucible were not always easy to discern.

Temperature differences among the three methods of glass plate thermal ashing contributed to the different overall ranking, in my estimation, of these methods: method E (500°C), 16; method D (400°C), 18; and method F (600°C), 22 (see Table 5). Thermal ashing at 500°C, with the lowest relative ranking (lower values indicating more desirable methods), has the greater value as a method. Viewed in air, the carbonized fibro-vascular bundles exhibited limited exposure of the phytolith configuration and morphology, and the microscope focus needed constant adjustment. In deionized water, the files of silica phytoliths of *Musa* became apparent, though they were, at times,

**Table 5.** Summary matrix of methods evaluated in the study, sorted by method.

| method | safety | set-up $ | $ per sample | $ ten cycles | concurr. process | access to equip. | equip. operation | phytolith position | phytolith morph. | quan. or qual. |
|---|---|---|---|---|---|---|---|---|---|---|
| A) Mechanical Processing | 1 | 1 | 4 | 3 | 2 | 1 | 1 | 1 | 1 | 1 |
| B) Chemical Digestion | 2 | 3 | 5 | 2 | 1 | 2 | 2 | 5 | 1 | 1 |
| C) Thermal Ashing, Crucible | 1 | 4 | 3 | 4 | 1 | 2 | 3 | 4 | 4 | 3 |
| D) Thermal Ashing, Glass Plate, 400°C | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 3 | 2 |
| E) Thermal Ashing, Glass Plate, 500°C | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 1 |
| F) Thermal Ashing, Glass Plate, 600°C | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 3 | 4 | 4 |
| G) Oxygen Plasma Ashing | 1 | 5 | 1 | 5 | 2 | 3 | 1 | 2 | 1 | 1 |

| | | | | |
|---|---|---|---|---|
| most desirable 1 | 2 | 3 | 4 | least desirable 5 |

**Note.** The best method is not necessarily the one with the lowest total score (i.e., mechanical processing, or thermal ashing between glass plates at 500°C), rather, the best method or methods must be based on the criteria that are most important to the researcher. For instance, if *cost per sample* criterion is the most important, then oxygen plasma ashing is the *first* choice, however, if the *set-up cost* is most important, then oxygen plasma ashing is the *last* choice. Multiple criteria may contribute to deciding which methods are chosen for a study. Once a set of criteria are determined, they must be prioritized, then the table can be repeatedly sorted, beginning with the least important criteria and moving to the most important criteria.

encrusted with carbonized material. In glycerol, the silica bodies became less visible than they had been in deionized water[13]. Thermal ashing at 400°C, provided less data than thermal ashing at 500°C, because the lower temperature resulted in a lower degree of ashing, thus there was more carbonized debris to confuse the viewing process. Thermal ashing at 600°C, was valued lower than thermal ashing at 500°C or 400°C, because the higher temperature managed to distort the silica files, and calcium oxalate raphide crystals of *Musa*. The silica files and contents of a raphide sack appeared eroded; for example, sample 0011a contained a raphide sack full of fused raphide shards. In addition, the calcium oxalate crystals appeared tarred, rather than clear. Plate 1 illustrates the results of thermal ashing: photomicrograph a) and scanning electron micrograph b) show the residue of thermal ashing at 600°C. The fusing of the silica surface is apparent in the photomicrograph, and very apparent in the scanning electron micrograph (the surface of the silica units is granular and the form is domed, when thermal degrdation has not occurred). Photomicrograph c), Plate 1, shows the darkened, bent, and broken quality of the calcium oxalate crystals from *Agave*. Thermal ashing at 600°C was additionally problematic because of distortions of the glass plates, and the scattering of the ash that occurred.

The yields from oxygen plasma ashing were good, because they provided an exposed version of the phytoliths, in place, as well as a view of the fibres in a degraded state that differed from the thermal or chemically degraded samples (see Appendix D). Oxygen plasma ashed sample residues were difficult to handle due to their fragility. Removal of the ashed sample from the reaction chamber, mounting and handling, all potentially compromised the positioning of the residue on the glass plate. The best results were achieved when the glass plate was removed from the outlet end of the chamber (which minimized electrostatic disruption by the charged coil at the inlet end), observed, and recorded immediately.

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[13] Glycerol worked well as a mountant for samples that contained only calcium oxalate crystals and no silica.

**PLATE 1.**

**PLATE 1.**

**a)** Photomicrograph of *Musa textilis* Née (abaca, or Manila hemp), WS grade fibre, which has been thermal ashed between glass plates at 600°C. The image shows the alignment of multiple files of silica bodies, or units, however, the units are fused and distorted.

**b)** Scanning electron micrograph of residue from *Musa textilis* Née (abaca, or Manila hemp), WS grade fibre, which has been thermal ashed between glass plates at 600°C. The micrograph shows the smooth, fused surface of a silica unit. The characteristic grainy surface is absent, and the sinuate border has been reduced to a scalloped edge.

**c)** Photomicrograph of *Agave* (species not determined[14]), which has been thermal ashed between glass plates at 500°C. The micrograph shows the alignment of the calcium oxalate styloid crystals with the fibre bundles, and it also shows the darkening, bending and breaking that occurs with thermal ashing. One can see from the slide why Catling and Grayson (1982) said the crystals resembled "small black bananas" (p. 56).

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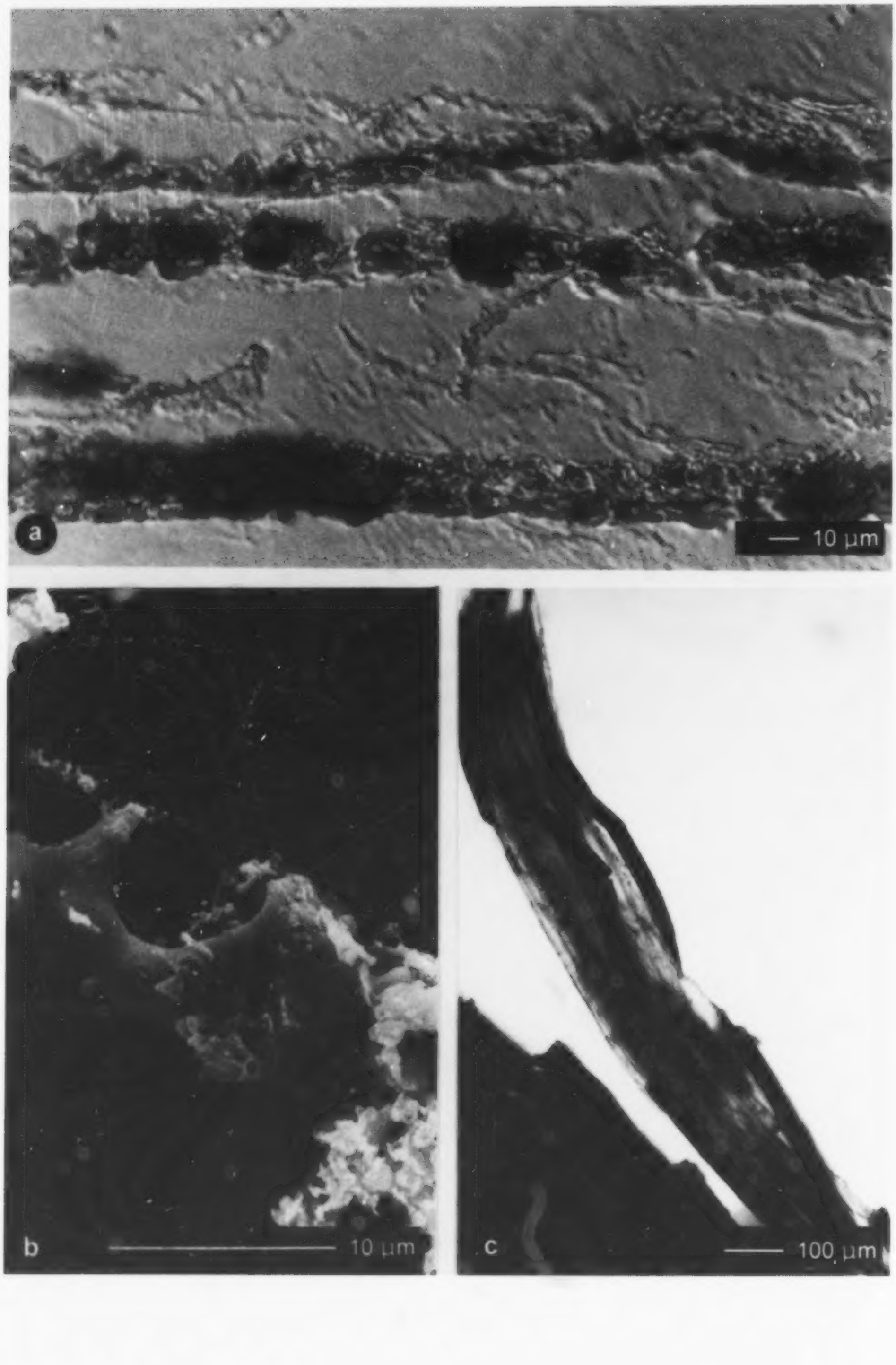
[14] The species is either *Agave cantala* (maguay), or *Agave fourcroydes* (henequen), as is discussed in the *Materials and Methods* section.


a
10 µm
b
10 µm
c
100 µm

### Discussion Of The Seven Phytolith Distillation Methods

Working with multiple methods on a set of samples nets the best range of data for each specimen, however, choosing the best methods for a particular material depends on the requirements of the researcher. Table 5 is a summary of Table 4, the method/criteria matrix, but with the ranking of the methods expressed numerically. The numbers express the relative value of each method for each criterion (1 being most desirable, and 5 being least desirable), and not the absolute value. For some researchers the only criterion of the treatment is that it isolates phytoliths with good morphology. In that instance, the method of choice would be chemical digestion. In another example, if the *cost per sample* criterion is the most important, then oxygen plasma ashing would be the first choice, however, if the *set-up cost* is most important, then oxygen plasma ashing would be the least desirable choice. Multiple criteria may contribute to deciding which method(s) are chosen for a study. Once a set of criteria is determined, each criterion must be prioritized, and, then, the table of values can be repeatedly sorted, beginning with the least important criterion and moving to the most important criterion.

Table 6 illustrates an example of criteria prioritizing and sorting, in order to select an appropriate set of methods for a research purpose where set-up cost, access to equipment, equipment operation and quantitative or qualitative data were not limiting criteria. For the thesis study, *set-up cost*, *access to equipment*, *equipment operation*, and *quantitative or qualitative data* were first deemed to be not significant criteria; they were given a zero relative value. The remaining criteria were prioritized as follows: *retention of phytolith morphology*, *retention of phytolith positioning within the plant sample*, *time to complete ten cycles*, *ability to process samples concurrently*, *safety*, and *cost per sample*. The *cost per sample* was the least significant criterion, thus, in an effort to not skew the results, the relative values were discretionally divided by 2. The third step in the process was to sort by criteria, from least significant to most significant. A very important point to recognize is that the calculated total of the method does not necessarily indicate that the

**Table 6.** Matrix illustrating the results of criteria prioritizing, and sorting in order to select the most desirable methods for use in the current study.

| Method | safety | set-up $ | $ per sample | ten cycles | concurr. process | access to equip. | equip. operation | phytolith position | phytolith morph. | quan. or qual. |
|---|---|---|---|---|---|---|---|---|---|---|
| A) Mechanical Processing | 1 | 0 | 2 | 3 | 2 | 0 | 0 | 1 | 1 | 0 |
| G) Oxygen Plasma Ashing | 1 | 0 | 0.5 | 5 | 2 | 0 | 0 | 2 | 1 | 0 |
| B) Chemical Digestion | 2 | 0 | 2.5 | 2 | 1 | 0 | 0 | 5 | 1 | 0 |
| E) Thermal Ashing, Glass Plate, 500°C | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| D) Thermal Ashing, Glass Plate, 400°C | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 3 | 0 |
| F) Thermal Ashing, Glass Plate, 600°C | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 3 | 4 | 0 |
| C) Thermal Ashing, Crucible | 1 | 0 | 1.5 | 4 | 1 | 0 | 0 | 4 | 4 | 0 |

| | | | | |
|---|---|---|---|---|
| most desirable 1 | 2 | 3 | 4 | least desirable 5 |

**Note.** Step one: *set-up cost*, *access to equipment*, *equipment operation*, and *quantitative or qualitative data* were deemed to be not significant criteria; they were given a zero relative value. Step two: the remaining criteria were prioritized from most to least important as follows: 1) *retention of phytolith morphology* (the highlighted column), 2) *retention of phytolith positioning within the plant sample*, 3) *time to complete ten cycles*, 4) *ability to process samples concurrently*, 5) *safety*, and 6) *cost per sample*. In this example, *cost per sample* was the least significant criterion, therefore the relative values were discretionally divided by 2. Step three: the criteria were sorted, from least significant to most significant. In this example, the sorting process indicated that the four most viable methods would be mechanical processing, oxygen plasma ashing, chemical digestion, and thermal ashing between glass plates at 500°C, in that order, despite the totals indicating a different order (see discussion in this section of the thesis).

method is most valuable. The most valuable method results from the sorting by criteria. The final sorting of the criteria was by way of the *retention of phytolith morphology* criterion, and, therefore, it is highlighted, rather than shaded on Table 6.

The result of the sort indicated that the four most viable methods would be mechanical processing, oxygen plasma ashing, chemical digestion, and thermal ashing between glass plates at 500°C. These are the same four methods of preference that are evident in the literature review, and they were the methods chosen for phase two of the research, the treatment of plant organ or tissue samples other than *Musa textilis*.

It is important to note here that the value given to each criterion for each method was an independent process. Additionally, the value scale within criterion varies, based on the relative differences of value between methods (in one case two steps are used, in some cases three steps are used, and in other cases four or five steps are used). The total value for each method was therefore assigned with minimal subjective input, as a consequence of not being able to tabulate the results until the matrix was complete. Each researcher must decide where the limitations are: equipment, time, finances, etc.

The surprises in the evaluation of the methods were the relative second place standing for method G, oxygen plasma ashing, and the last place standing for method C, thermal ashing in a crucible. Oxygen plasma ashing takes a long time to complete, however, the yield is valued, and the cost to process one sample is low. Crucible thermal ashing may have had a better showing if the quantification of the ash had included the quantification of the phytoliths on their own.

### Discussion Of Imaging Processes

The imaging of the phytoliths distilled from the four methods of choice was done with manual micrographs (drawings), photomicrographs, scanning electron (SE) micrographs, which included energy dispersive x-ray analysis (EDXA), and confocal laser scanning (CLS) micrographs (see the *Methods and Materials* section). The mechanically processed samples were viewed and recorded successfully using all means. Except for one *Musa* sample, the plasma ashed samples were too fragile to be viewed by any means except light microscopy in a mountant of air. As a result, wide strip, or WS grade *Musa* is the only specimen for which there are SE micrographs of plasma ashed samples. The chemically ashed samples were recorded on manual micrographs (drawings), photomicrographs (including polarized light micrographs), and CLS micrographs. As a consequence of eventually mounting all but one of the chemically ashed sample residues in glycerol, there was only the one to image using SEM. The glass plate ashed samples (the final set of data) were imaged using manual micrographs, photomicrographs, and SE micrographs. In particular, a photomicrograph was made of a *Musa* sample, to show the degradation of the silica files that occurred during glass plate ashing at 600°C.

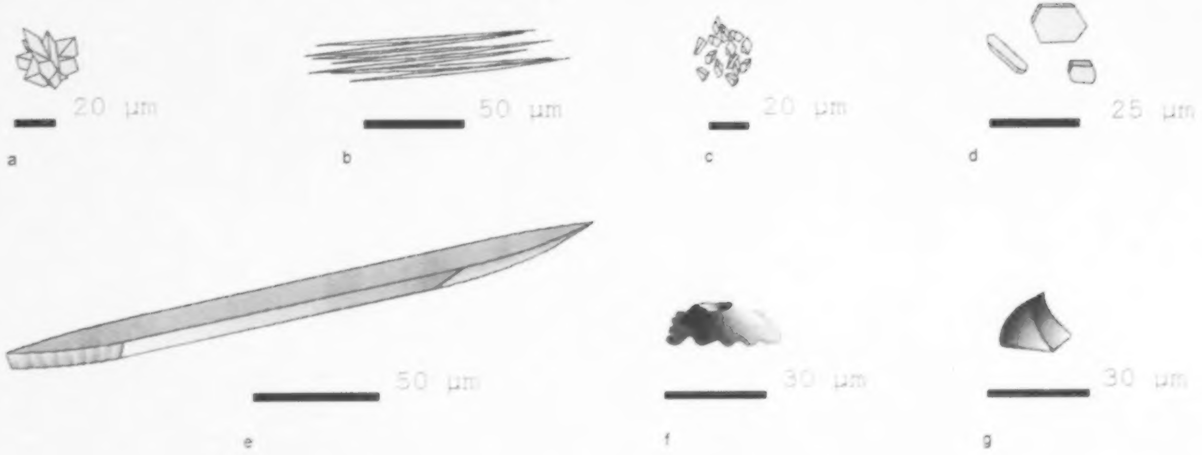
The most satisfactory results were the SE micrographs. Once a sample had been successfully coated in gold and entered into the reaction chamber, the working distance, voltage, and magnification were manipulated to reveal information about the relationship between phytoliths and fibre bundles, and enabled an accurate description of the phytolith morphology. The scale feature (indicated on the SE micrographs), the ability to determine atomic species present (EDXA), and the sequential recording of the selected images on black and white film, and digital disc further enhanced the process.

The least used process was CLSM, in part due to the nature of the distilled residues, and in part due to my resistance to using fluorochromes to make the silica bodies and calcium oxalate crystals respond to the wavelength of laser light that was being used. None-the-less, the few CLS micrographs that were made were useful.

### Phytolith Data

Definitive phytolith data were collected for all the specimens in the study, except for *Boehmeria nivea*. *Musa textilis*, *Musa* (species not defined), and *Cannabis sativa* contained silica bodies, and all of the specimens contained calcium oxalate crystals, in one or more of the full spectrum of forms: crystal sand, raphide crystals, styloid crystals, rhomboid crystals, and druse crystals. Figure 3 illustrates the forms of phytoliths that

**Figure 3.** The unit forms of silica and calcium oxalate phytoliths observed during the research.



**Note.** Forms of phytoliths: a) calcium oxalate druse crystal, in *Musa* and *Cannabis*; b) calcium oxalate raphide crystals, in *Musa* and *Agave*; c) calcium oxalate crystal sand, in *Musa* and *Cannabis*; d) calcium oxalate (and possibly magnesium phosphate in *Musa*) rhomboid crystals, in *Musa*, *Cannabis*, and *Corchorus*; e) calcium oxalate styloid crystal, in *Agave*; f) silicon dioxide body, in *Musa*; g) silicon dioxide trichome base, in *Cannabis*.

were observed in different samples examined; Figures 4 through 8 are the energy dispersive x-ray analyses of the phytoliths examined with scanning electron microscopy; and Table 7 summarizes the phytolith data that were observed and compares the research findings with those of other researchers.

**Figure 4.** EDXA of a silica body from *Musa textilis* Née (**abaca**, or **Manilla hemp**).

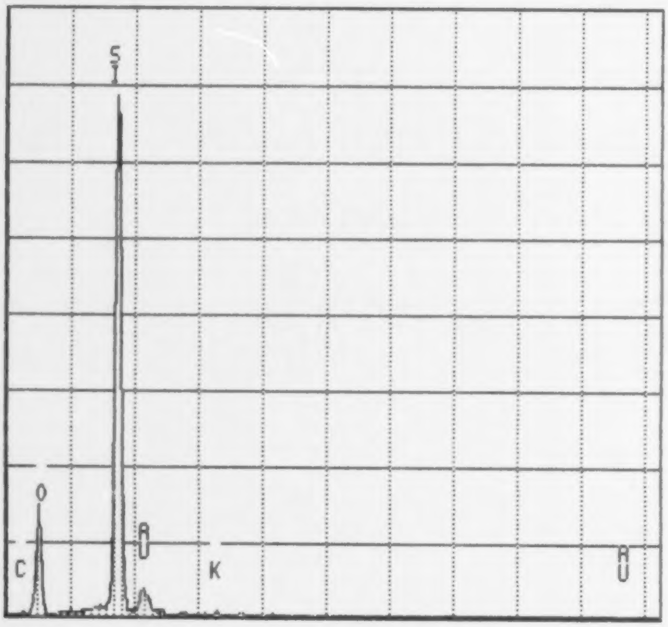


**Figure 5.** EDXA of a rhomboid crystal from *Musa textilis* Née (**abaca**, or **Manilla hemp**).

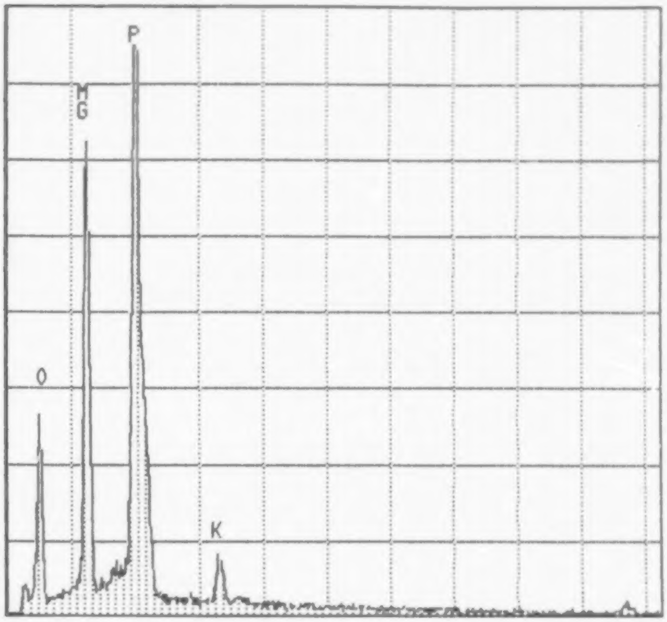


**Figure 6.** EDXA of a styloid crystal from *Agave* (*cantala*, or **maguay**; or *fourcroydes*, or **henequen**).

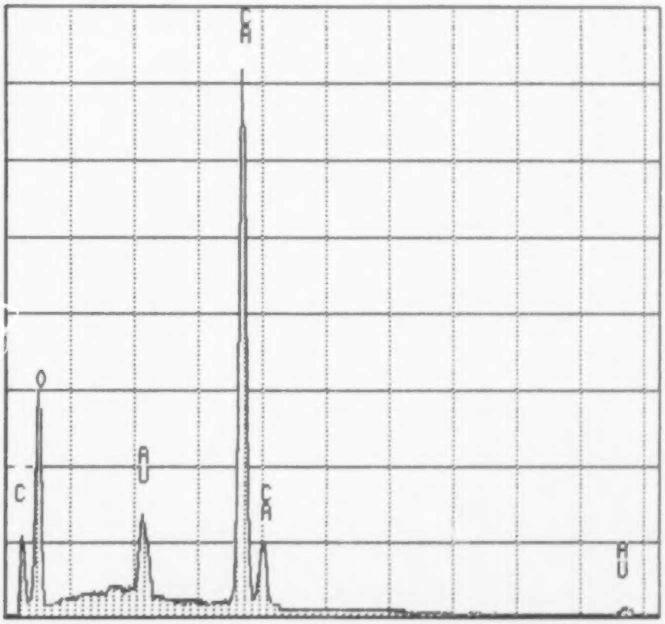


**Figure 7.** EDXA of a rhomboid crystal from *Corchorus capsularis* L. (**jute**).

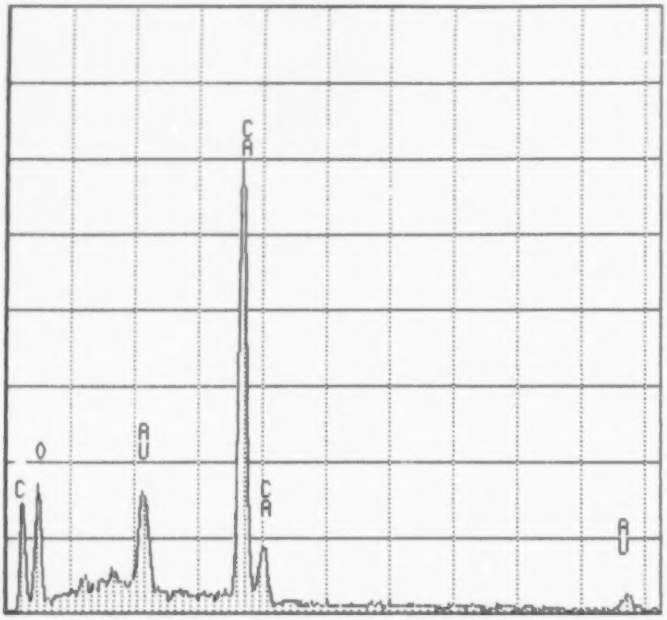


**Figure 8.** EDXA of a druse crystal from *Cannabis sativa* L. (**hemp**).

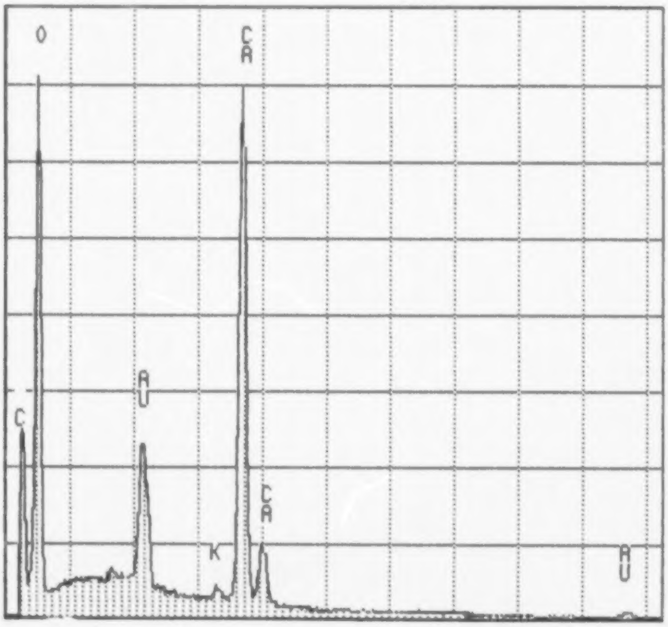


**Table 7.** Summary of phytolith data collected during the current research, as well as phytolith data reported in the literature for the species studied in the current research.

| MATERIAL | RESEARCHER(s) | PHYTOLITH CHARACTER | LOCATION OF PHYTOLITH, and other information. |
|---|---|---|---|
| ***Musa textilis*** **Née (abaca, or Manila hemp)** | ***Current Study*** | **Silica Body** | **Multiple files located adjacent to fibre & fibro-vascular bundles; square to rectangular bases, with asymmetrical open-domed protrusion; units contained in cells, linked via plasmodesmata to fibre bundles and to each other; silicification occurs in the plasmodesmata, giving the units a serrated look when they are viewed individually; when container cells ashed, units cling together; units disaggregate in chemically digested samples.** |
| | Catling and Grayson, 1982 | | Quoting Tomlinson (1959), located in abaxial epidermis, "small, irregularly spherical silica bodies"; near vascular bundles, silica bodies that "are rectangular, often with a central depression" (p. 59); no illustration. |
| | Florian, 1990 | | Location not indicated; "rows of silica cells, each cell with an asymmetrically placed circular silicon body" (p. 46). |
| | Goldberg, 1989 | | Location not indicated; presence of silica bodies (p. 59); no illustration. |
| | Piperno, 1988 | | Located in epidermal tissues (p. 252); forms are different than found in Heliconia; *Musa* with deeper, wider troughs, Heliconia with raised, shallow troughs; bodies are "at least genus specific" (p. 65). |
| | Schaffer, 1991a | | Location not indicated; uses the term "stegmata (silicified cells), e.g., $SiO_2$"; confirmatory test for silica is its red appearance when mounted in a mixture of phenol and clove oil (p. 124). |
| | Tomlinson, 1959 | | "Stegmata (Figs. 6, 8, 10, 11) present in longitudinal files adjacent to the fibrous portions of the bundle-sheaths in the lamina . and less frequently in the corresponding position in the leaf stalk, the walls of the silica-cells adjacent to the fibres being slightly thickened and pitted"; "more or less rectangular, trough-like and each with a central depression away from the fibres"; portions of the silica bodies "project into pits in the thickened part of the walls" (p. 794); illustration shows the association of the silica files with fibres |

| | | | |
|---|---|---|---|
| | **Current Study** | **Calcium Oxalate Raphide Crystals** | **Located in sacs and as individual liberated units on the aerenchyma surface, which spans the abaxial and adaxial surfaces of the leaf sheath, between vascular ribs; containment sac does not resist chemical digestion.** |
| | Catling and Grayson, 1982 | | Raphides in "mesophyll, near the adaxial epidermis" (p. 63). |
| | Goldberg, 1989 | | Location not indicated; presence of raphides mentioned (p. 59). |
| | Florian, 1990 | | No mention of raphides, but mention of silica bodies. |
| | Franceschi and Horner, 1980 | | Referencing Lott (1976), located on the surface of aerenchyma. |
| | Lott, 1976 | | Located on the "diaphragm" (p. 32) of aerenchyma tissue, between petiole ribs; idioblasts are morphologically different (larger) than neighbouring cells |
| | Tomlinson, 1959 | | Location not indicated (aerenchyma not mentioned); raphide sacs numerous. |
| | ***Current Study*** | **Rhomboid Crystals (possibly magnesium phosphate)** | **Located in abundance, in or near the epidermis in samples of all *Musa* materials that were examined; birefringent; EDXA indicates presence of P, Mg, K, and O; basic form appears in many dimensions.** |
| | Tomlinson, 1959 | | Specific location not indicated; writes of the "crystals of calcium oxalate present as abundant rhombohedra" (p. 794). |
| ***Musa* (species not defined)** | Wilson, 1985 | Silica Body | "unusual, silicified short cell is probably aligned lengthwise with non-silicified long cells and vein segments of the epidermis of the banana's leaves"; one image of a single body (p. 91). |
| ***Agave* (various species, *cantala*, maguay; *fourcroydes*, henequen; & *sisalana*, sisal)** | ***Current Study*** | **Calcium Oxalate Styloid Crystal** | **Found in sacs adjacent to the fibre bundles, and in the ground tissue surrounding the fibre bundles; sacs adjacent to the fibre bundles are connected via plasmodesmata; sacs resist chemical digestion; thermal ashing bends crystals and coats crystals with tar.** |
| | Arnott, 1973 | | Location not indicated; cell wall collapses around crystals; illustration of three crystals in one sack, in transverse section (p. 620). |
| | Catling and Grayson, 1982 | | Crystals in parenchyma; not shown near fibres in drawings, but one fibro-vascular bundle shows characteristic "boxed" void from where a crystal has been displaced; "in every ashed sample, there are large numbers of crystals"; "crystals are curved and resemble small black bananas" (p. 56). |

| | | | |
|---|---|---|---|
| | Florian, 1990 | | Location not indicated; uses the term "birefringent rod-shaped calcium oxalate crystals" (p. 48) |
| | Franceschi & Horner, 1980 | | Located in mesophyll. |
| | Gentry, 1982 | | No mention of styloids. |
| | Goldberg, 1989 | | Mention of raphides as commonly present; no mention of styloids (p. 42). |
| | Jones and Bryant, 1992 | | Location not indicated; found "non-druse 'exotic' crystals"; "were unable to determine the origin of these exotic-type crystals" (p. 236). |
| | Schaffer, 1991 | | Location not indicated; uses the term "rod-like crystals", and indicates that it is calcium carbonate (p. 124) |
| | Wattendorff, 1976a | | Found in "the tissue" (vague) in "cell, or sack"; sac is resistant to acids; some sacs contain "a few" crystals; called styloids or pseudoraphides (p. 163). |
| | Nobel, 1988; 1994 | | No specific mention of styloids in *Agave*, though does mention presence of calcium oxalate in *Agave*, and calcium oxalate crystals in cacti (p.177). |
| | ***Current Study*** | **Calcium Oxalate Raphide Crystal** | **Found in sacs in the ground tissue adjacent to fibre bundles; sac is resistant to chemical digestion; thermal ashing fuses crystals and coats crystals with tar.** |
| | Arnott, 1973 | | Located in leaf "mucilaginous exudate" (p. 609); cell wall collapses around crystals; raphide crystals are reported to cause contact dermititis. |
| | Catling and Grayson, 1982 | | Reference to styloids, but no mention of Raphides |
| | Florian, 1990 | | Reference to styloids, but no mention of Raphides |
| | Franceschi and Horner, 1980 | | Located in mesophyll. |
| | Goldberg, 1989 | | Location not indicated. |
| | Jones and Bryant, 1992 | | No mention of raphides, but do mention, but not locate, "exotic-type" (styloid) crystals (p. 236). |
| | Nobel, 1988; 1994 | | No specific mention of raphides in *Agave*, though does mention presence of calcium oxalate in *Agave*, and calcium oxalate crystals in cacti (p.177). |
| | Schaffer, 1991 | | Reference to styloids, but no mention of Raphides |
| | Wattendorff, 1976b | | Found in plant tissues (vague) in "cell, or sac"; sac is resistant to acids(p.303). |
| ***Boehmeria nivea*** **(L.) Gaud. (ramie)** | ***Current Study*** | **Calcium Oxalate Druse Crystals** | **Study used fibre ultimates, which contained occasional definite crystals, and some fragments.** |
| | Bozarth, 1993 | | No report of druse crystals, but did report a type of "stalked verrucate" (p. 96) silicon dioxide inclusion, or phytolith. |
| | Florian, 1990 | | Mention of *Boehmeria*, no mention of crystals (p. 51); mentions "rows of crystal druses present in parenchymous tissue" of *Urtica dioica L.* (stinging nettle, in family with *Boehmeria*) (p.51) |

| | | | |
|---|---|---|---|
| | Catling and Grayson, 1982 | | Illustration shows files of druse crystals (p. 33); "cluster <druse> crystals are seen in ordinary parenchyma cells and in chambered cells" (p. 34). |
| | Metcalfe and Chalk, 1950 | | "Solitary" (druse) crystals abundant; rare presence of raphides and "solitary" (rhomboid) crystals mentioned; location not mentioned (p. 1345). |
| | Schaffer, 1991a | | Location not indicated; uses the term "cluster crystals" and indicates that it is calcium carbonate (p. 124). |
| ***Corchorus capsularis*** **L. (jute)** | ***Current Study*** | **Calcium Oxalate Rhomboid Crystals** | **Found attached to fibre bundles; appear to be contained in a sac; near nodes in some examples.** |
| | Catling and Grayson, 1982 | | Illustration of *Corchorus* olitorius showing file of rhomboids adjacent to fibre bundle, and "cluster" (druse) crystal in the cortical parenchyma; "chains of rhombic crystals and individual cluster <druse> crystals" found in residue from ashed samples; "occasionally, one or two cluster crystals are mixed in a chain of rhombic crystals" (p. 41). |
| | Florian, 1990 | | Mention of *Corchorus*, but no mention of crystals (p. 49). |
| | Metcalfe and Chalk, 1950 | | Location not indicated; abundant "cluster" (druse) crystals; noted presence of "solitary" (rhomboid) crystals. |
| | Jarman and Kirby, 1955 | | Found in the "parenchyma associated with the ultimate fibres" (p. 281); "distinguishing feature of jute is the occurance of chains of solitary <rhomboid> crystals"; "tendency in jute for solitary crystals to accumulate in the region of the nodes" (p. 285). |
| | Schaffer, 1991a | | Location not indicated; uses the term "solitary crystals", and indicates that it is calcium carbonate (p. 124). |
| ***Cannabis sativa*** **L. (hemp)** | ***Current Study*** | **Calcium Oxalate Druse and Rhomboid Crystals** | **Files of druse crystals, with occasional rhomboid elements, adjacent to primary phloem fibre bundles, and individual druse crystals to the periphery of the pith parenchyma, concentrated close to the xylary elements .** |
| | Catling and Grayson, 1982 | | Illustration shows druse crystals in ground parenchyma, and in occasional files adjacent to the fibre bundles; "cluster <druse> crystals occur singly or in chambered cells" occasional "rhombic or prismatic forms" (p. 23). |
| | Dayanandan and Kaufman, 1976 | | Location of calcium deposits "cystoliths" not defined; (though the researchers were looking mostly at leaves, they also were looking at other shoot tissues). |
| | Florian, 1990 | | Location not indicated; mentions that "crystals are present in the ash" (p. 49). |

| | | | |
|---|---|---|---|
| | Jackson, and Snowdon, 1974 | | Illustrates fragment of fibre bundle with associated "cluster" (druse) crystals in a file (plate 32); illustrates "cystoliths" (same as observed in the current study) as originating from the "upper epidermis of the bract" (plate 31). |
| | Metcalfe and Chalk, 1950 | | Druse crystals "plentiful in the phloem" and "in the peripheral part of the pith", crystals an important diagnostic characteristic (pp. 1256 - 1257). |
| | Schaffer, 1991a | | Location not indicated; uses the term "cluster crystals" and indicates that it is calcium carbonate (p. 124). |
| | ***Current Study*** | **Silica body** | **Silicified trichome bases, believed to be in epidermis.** |
| | Dayanandan and Kaufman, 1976 | | Silicified trichome bases, from epidermis. |
| ***Material Not Specified*** | Cheavin, 1938 | No reference to any species in the current study. | Recognizes that the crystals are deposited regularly along the veins, and near the "scelerotic cells" (p. 156) (fibre scereids). |

### ***Musa textilis*** **Née (abaca, or Manila hemp)**

One or more files, or columns of silica bodies were found parallel to 100% of the 77 fibre bundle samples from the leaf sheath, WS grade fibre, G grade fibre, and linen samples (see data in Appendix D, Tables D1, D2, D3, & D6). Individual silica units were found in 79% of the 34 samples in which the fibre bundles had been disaggregated, i.e.,in paper or pulp samples, and in chemically ashed samples (see data in Appendix D, Tables D1 through D5). Overall, 93% of the 98 *Musa* residues that were observed had troughed silica phytoliths.

The appearance of the silica files and individual silica bodies varied depending on the process that was used to distill them. Plate 2 illustrates three magnifications of a manually sectioned WS grade fibre. Micrograph a) shows a file of silica bodies aligned with the fibre bundle, as well as the "brickwork" remnants of previously adjacent parenchyma. The possibility exits that the seemingly discontinuous file of silica units seen in photograph a) was at one time continuous. Micrograph b) shows a higher magnification of the parenchyma cell remnants, and it shows the "sinuate" (Bombin, 1984, p. 57) edge and open-dome, or "troughed" (Piperno, 1988; Wilson, 1985) form of the silica units. In micrograph c) it is possible to see the granular texture of the silica units, even though they appear to be covered in a wax-like coating.

Plates 3, 4, and 5 illustrate examples of G grade *Musa textilis* fibres that have been cleared in chloral hydrate and critical point dried in preparation for scanning electron microscopy. In Plate 3, micrograph a) shows more than one file of silica units aligned with the fibre bundle; in this example it is possible to see a subtle counterclockwise twist of the fibre bundle (the silica file also follows the same twist). Micrograph b), a higher magnification of micrograph a), clearly illustrates the asymmetrical and random orientation of the open domed silica units; in this example it is also possible to see some

PLATE 2.

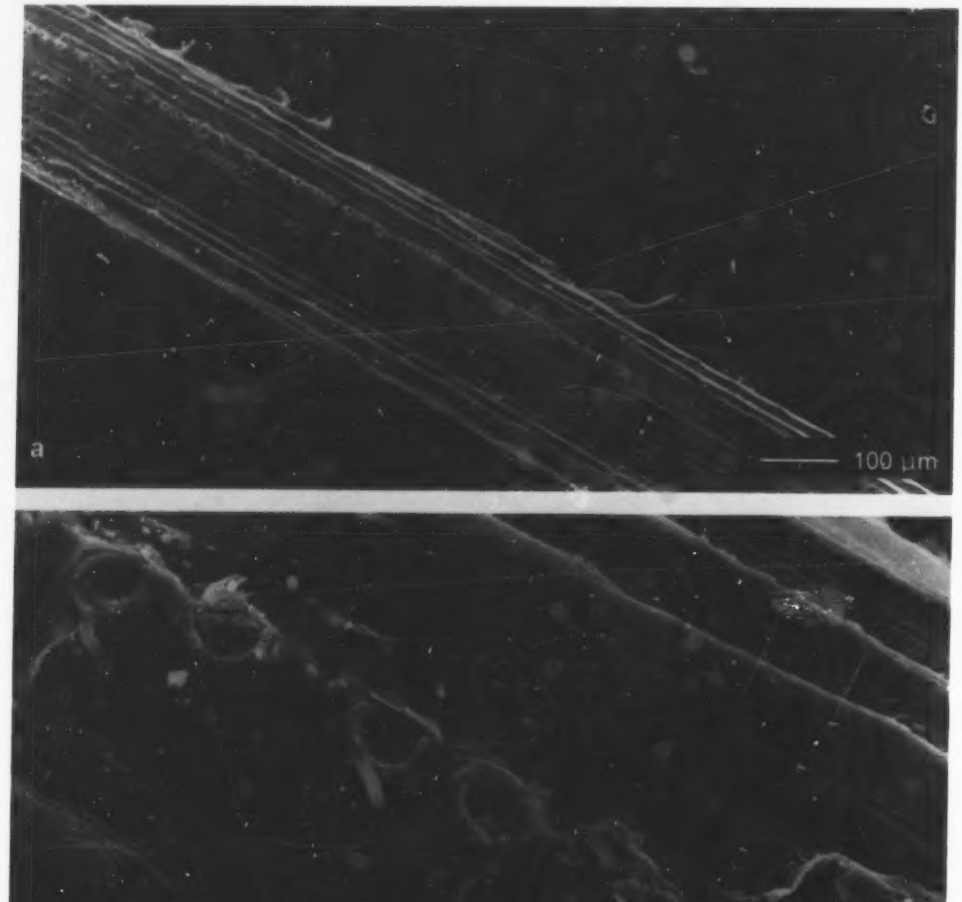
**PLATE 2.**

**Three scanning electron micrographs of *Musa textilis* Née (abaca, or Manila hemp), wide strip, or WS grade fibre.**

**a)** The image shows a file of silica units in alignment with a fibre bundle, as well as the "brickwork" remnants of previously adjacent parenchyma.

**b)** A higher magnification of the parenchyma cell remnants, and the "sinuate" (Bombin, 1984, p. 57) edge and open-dome, or "troughed" (Piperno, 1988; Wilson, 1985) form of the silica units.

**c)** In this image it is possible to see the granular texture of the silica units.


a
100 µm
b
10 µm
c
10 µm

# PLATE 3.

## PLATE 3.

**Three scanning electron micrographs of *Musa textilis* Née (abaca, or Manila hemp), G grade fibre, treated in chloral hydrate and critical point dried.**

**a)** The micrograph shows multiple files of silica units in alignment with the fibre bundle.

**b)** A higher magnification of a few silica units, as well as some fibre ends.

**c)** In this micrograph one can observe the characteristics of the sac, or lithocyst (Esau, 1965) that encloses the silica units, as well as the characteristic grainy texture of the silica units.

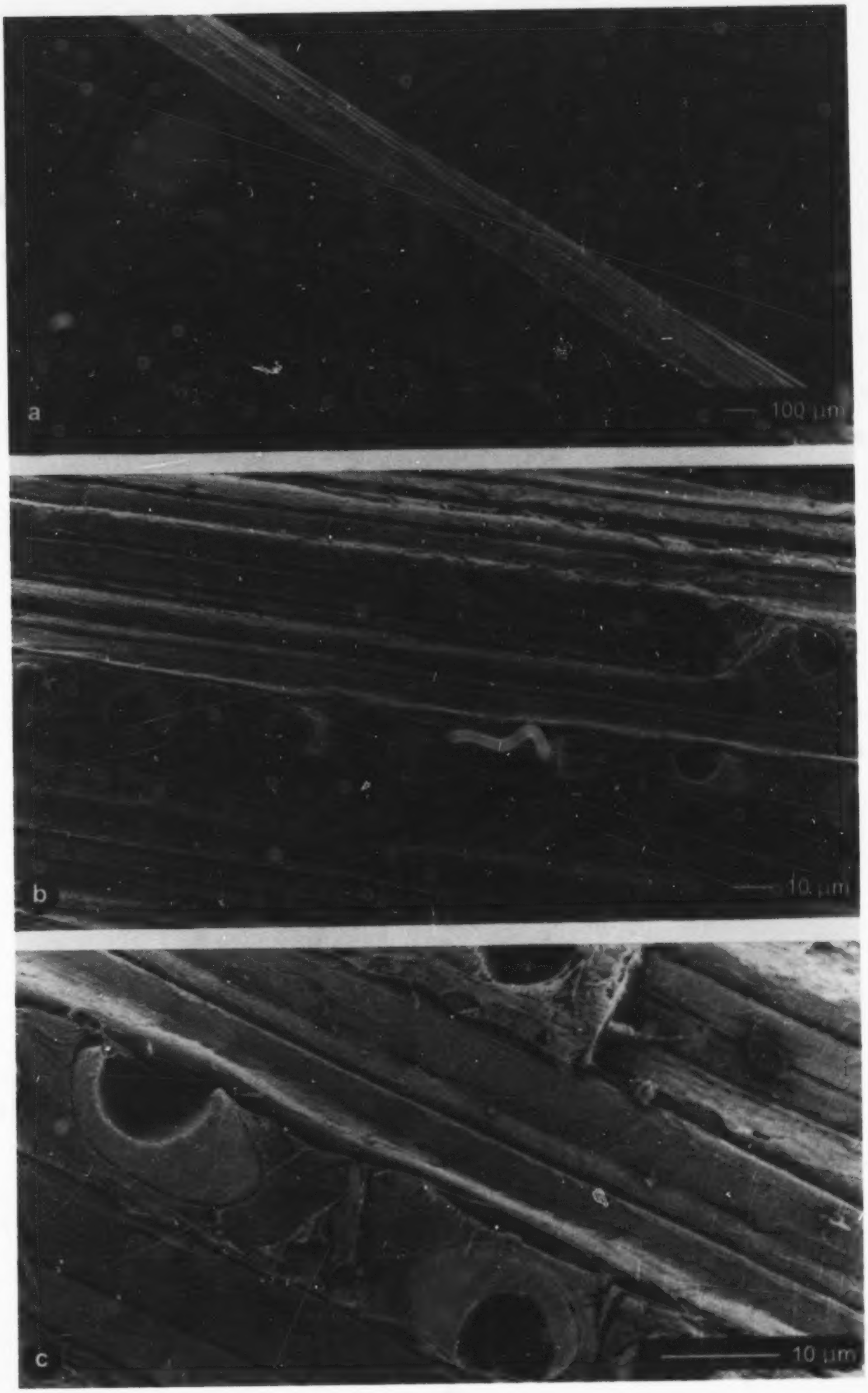

a
100 µm
b
10 µm
c
10 µm

**PLATE 4.**

**PLATE 4.**

**Three scanning electron micrographs of *Musa textilis* Née (abaca, or Manila hemp), G grade fibre, treated in chloral hydrate and critical point dried.**

**a)** The micrograph shows a file of silica units in alignment with the fibre bundle, as well as two fibre ends, flanking a third fibre.

**b)** The image shows a higher magnification of a few silica units, enclosed in sacs, or lithocysts (Esau, 1965).

**c)** In this example it is possible to see the arrangement of the macrofibrils of the lithocyst and of the adjacent fibres, as well as the silica body/lithocyst relationship, and the characteristic grainy texture of the silica units.

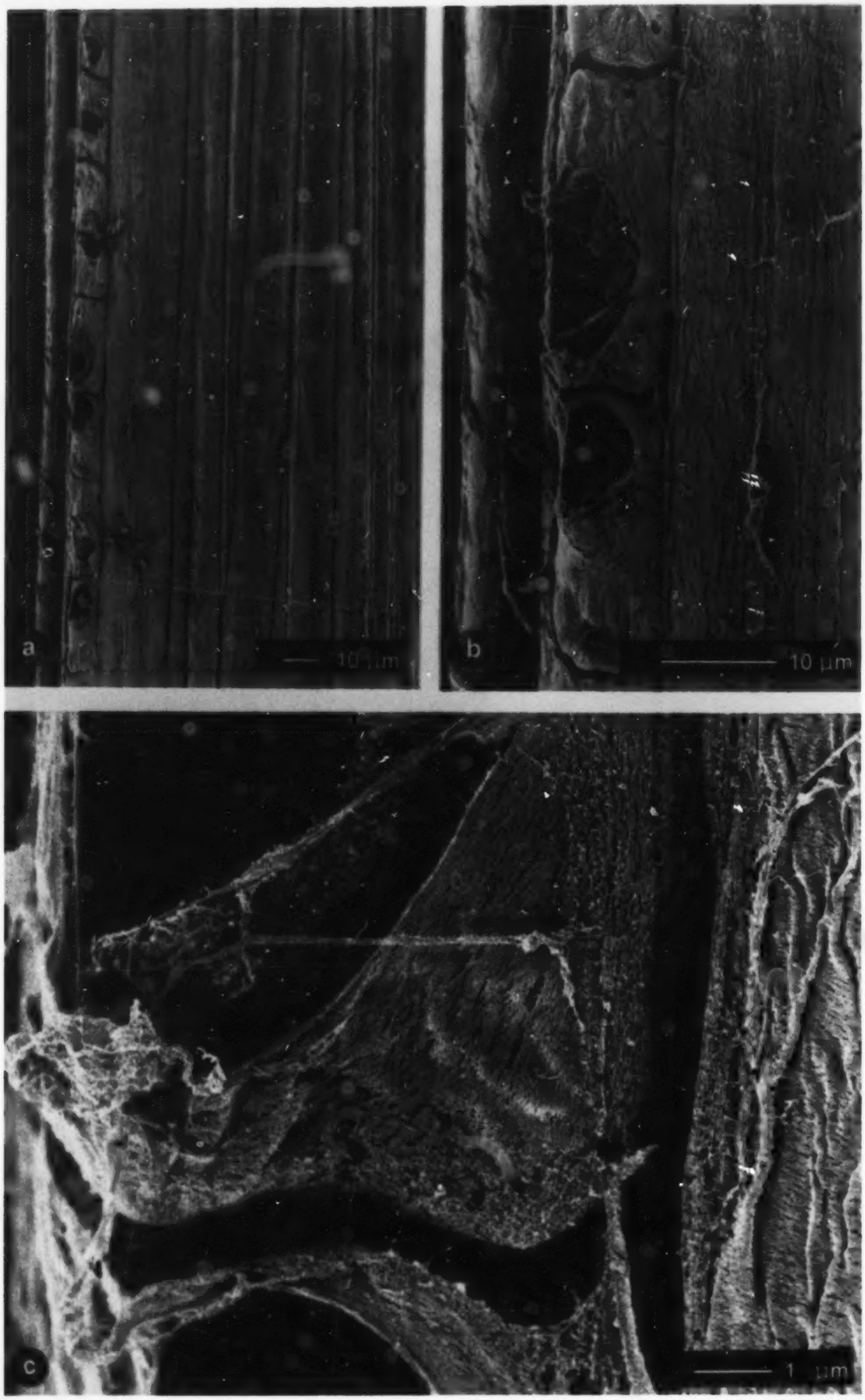
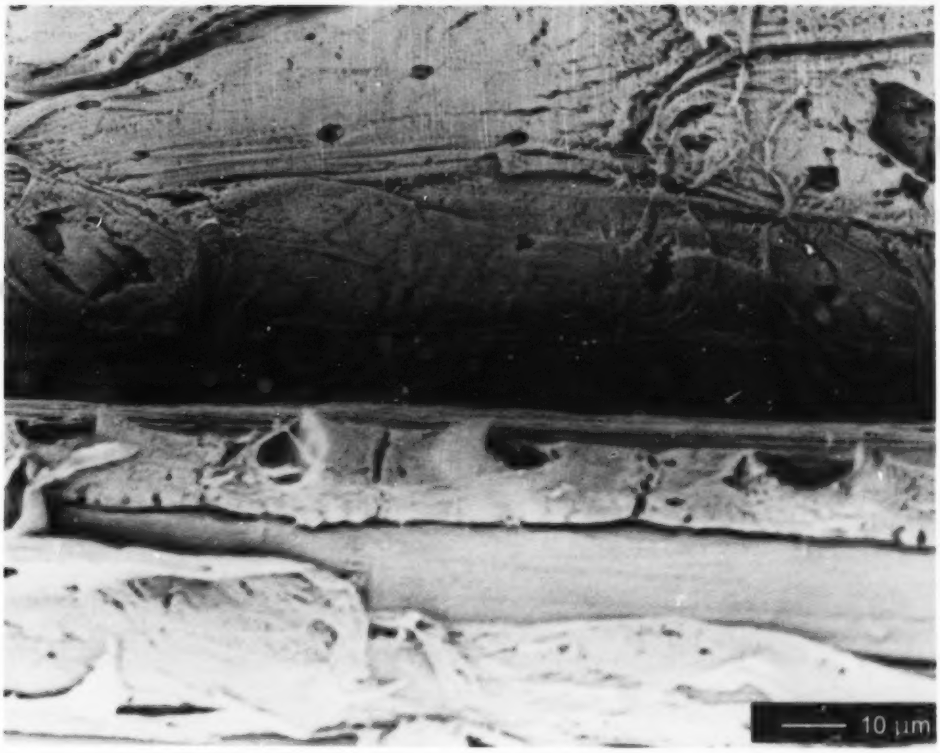

a
10 µm
b
10 µm
c
1 µm

PLATE 5.

**PLATE 5.**

**A scanning electron micrograph of *Musa textilis* Née (abaca, or Manila hemp), G grade fibre, which has been treated in chloral hydrate and critical point dried.**

This micrograph illustrates a detail of a fibre bundle that has some adhering parenchyma (bottom of micrograph) and epidermal tissue (top of micrograph). In this case it is possible to see the relationship of the silica units to the fibre bundle and to one another. In addition, it is possible to see that the silica units are not a part of the epidermis, but, rather, associated with the fibre bundle.


10 µm

fibre ends. In micrograph c), Plate 3, one can begin to observe the characteristics of the sac, or lithocyst (Esau, 1965) that encloses the silica units, as well as the characteristic grainy texture of the silica units. In the Plate 3 examples, as well as in the Plate 4 examples, the characteristic sinuate perimeter of the silica units is less visible due to the presence of the containment sac. Plate 4, micrograph c), illustrates a highly magnified version of the silica body/lithocyst relationship. In this example it is possible to see the fibrous nature of the lithocyst and of the adjacent fibres. Plate 5, again, shows an example of G grade fibre that has been cleared in chloral hydrate and critical point dried. In this example a fragment of adhering epidermal tissue can be seen adjacent to the fibre bundle; observing this image, one can understand how some researchers associate the silica bodies of *Musa* with the epidermis (Piperno 1988; Wilson, 1985).

Plate 6 shows examples of G grade fibres, as well as individual silica bodies, that have been oxygen plasma ashed. Micrograph a) illustrates the disruptions that can occur with the oxygen plasma ashing process, and, yet, it also shows the unique set of qualitative data that can be derived from the process. In the image it is possible to see the silica units in an orientation that is close to the original, and it is also possible to see the characteristic granular surface and sinuate perimeters of the silica units. Micrograph b), Plate 6, is a detail of the silica file from a). Micrograph c) is a view of two silica units from the fibre attachment side of the file, and one silica unit from the side of the file that faces away from the fibre bundle. In the micrograph it is possible to observe most of the salient characteristics of *Musa* silica units: the open domed top side; the sinuate borders (two that are flush with the perimeter of the fibre bundle, and two that are on the base of the silica unit, anchoring it to the fibre bundle); the asymmetrical placement of the trough; the variety of unit lengths; the regularity of unit width; and the grainy texture of the surface. Micrograph d) illustrates an upper (away from the fibre bundle) side of a single silica body, on which it is possible to see fracturing of the sinuate border.

Micrograph a), Plate 7, is a photomicrograph of a fibre that has been plasma ashed. The view that is obtainable with light microscopy is different than the view that is

# PLATE 6.

**PLATE 6.**

**Four scanning electron micrographs from a sample of *Musa textilis* Née (abaca, or Manila hemp), G grade fibre, which was oxygen plasma ashed.**

**a)** This micrograph shows the disruption that can occur during oxygen plasma ashing. It also shows the clarity of image that is possible with samples that have been oxygen plasma ashed, and subsequently viewed in the scanning electron microscope.

**b)** Detail of silica file on ashed fibre.

**c)** A view of two silica units from the fibre attachment side, and one silica unit from the side that faces away from the fibre bundle. In this micrograph it is possible to observe most of the salient characteristics of the silica units: the open domed top side; the sinuate borders (two that are flush with the perimeter of the fibre bundle, and two that are on the base of the silica unit, anchoring it to the fibre bundle); the asymmetrical placement of the trough; the variety of unit lengths; the regularity of unit width; and the grainy texture of the surface.

**d)** A view of the upper (away from the fibre bundle) side of a single silica body, on which it is possible to see fracturing of the sinuate border.

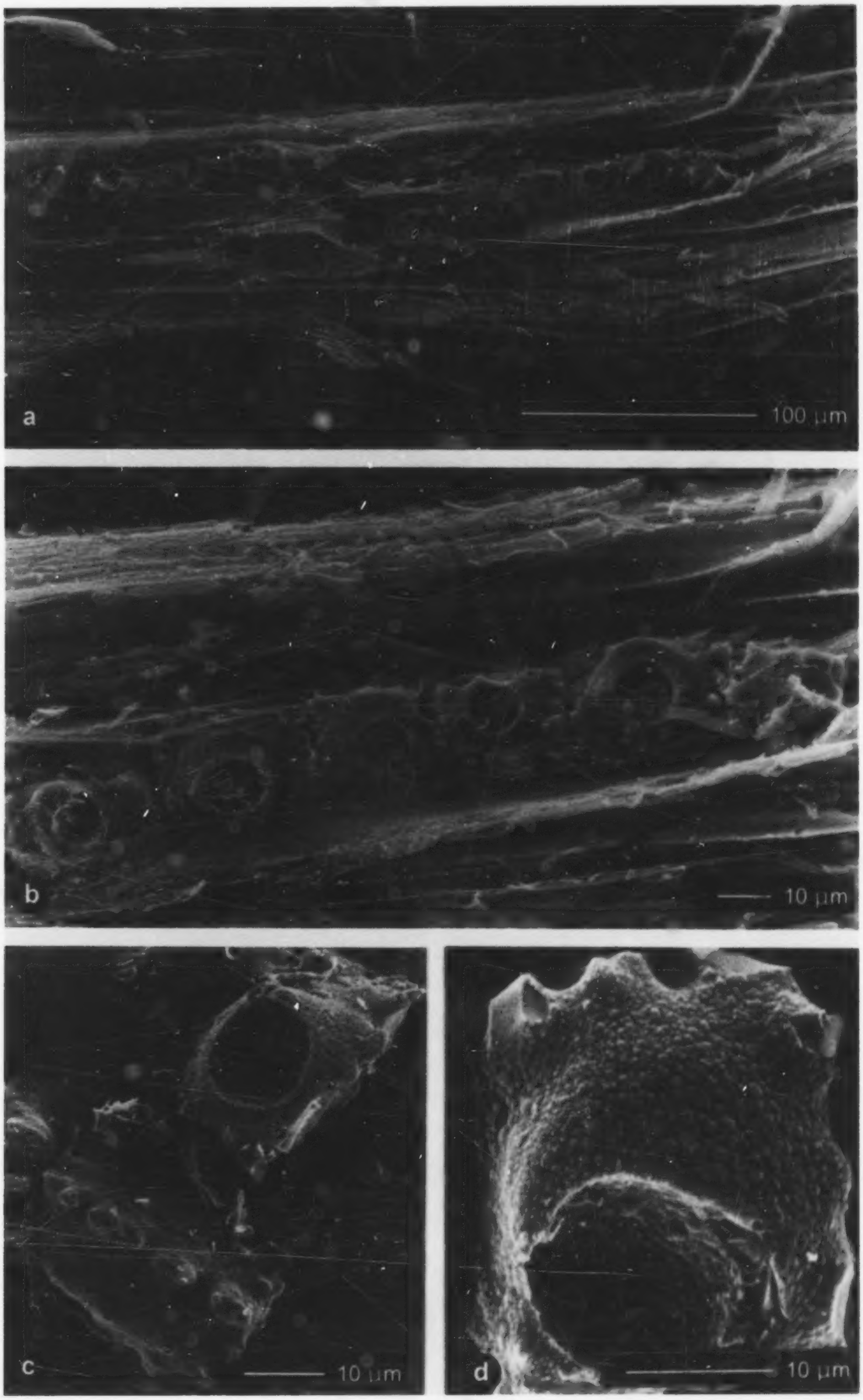

a
100 µm
b
10 µm
c
10 µm
d
10 µm

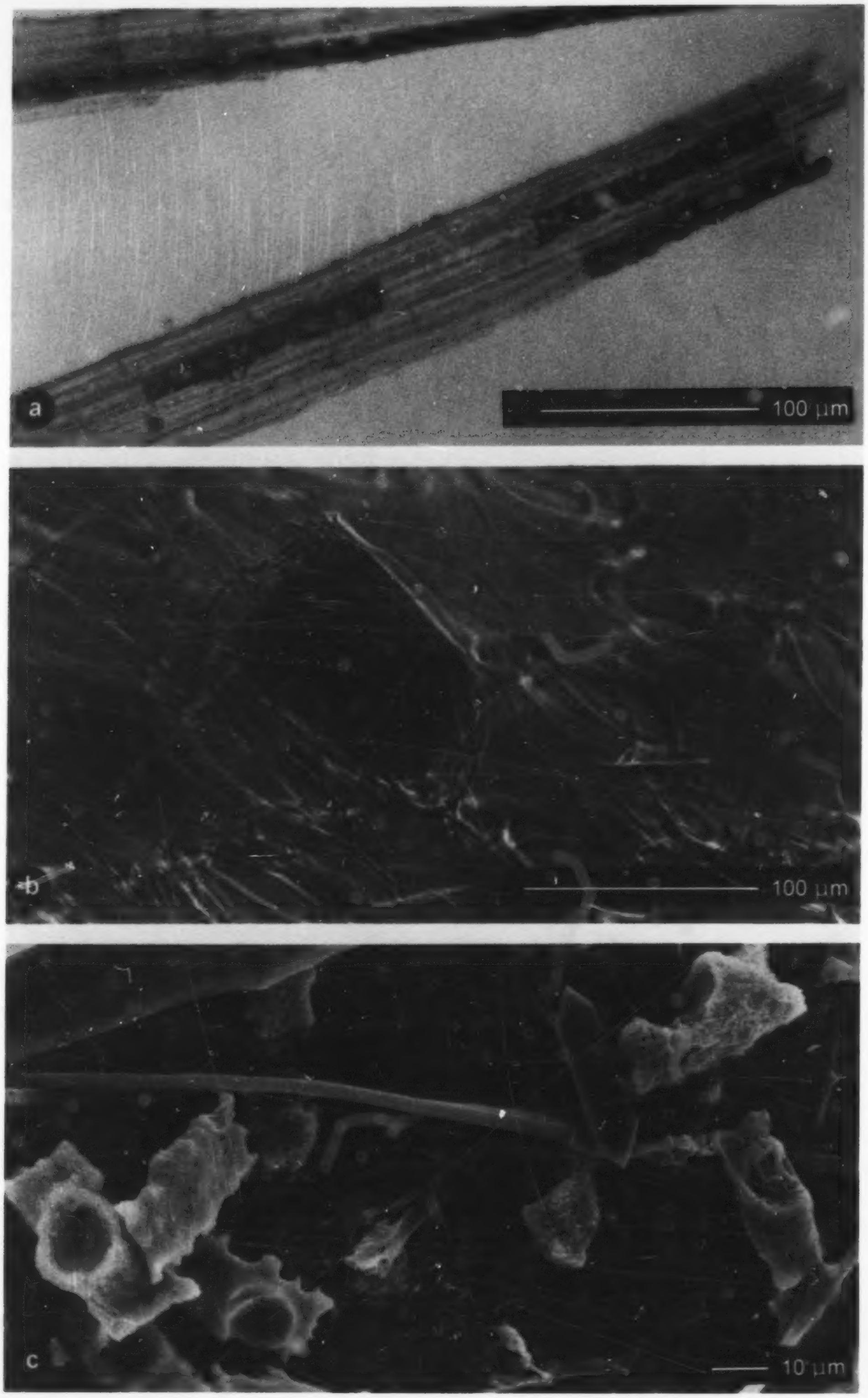
PLATE 7.

**PLATE 7.**

**a)** Photomicrograph of *Musa textilis* Née (abaca, or Manila hemp), G grade fibre, which was oxygen plasma ashed, showing the relationship of the silica files to the fibre bundle.

**b)** Photomicrograph of calcium oxalate raphide sac on aerenchyma tissue manually extracted from a specimen of *Musa textilis* Née (abaca, or Manila hemp) leaf sheath.

**c)** Scanning electron micrograph of residue from chemical digestion of a sample of *Musa textilis* Née (abaca, or Manila hemp) leaf sheath. Several silica bodies are present, and there is one rhomboid to right of the middle of the field, as well as a lactiferous element (organic resin deposit) in the upper left corner. The silica bodies are somewhat degraded, but the sinuate border and grainy surface are evident.

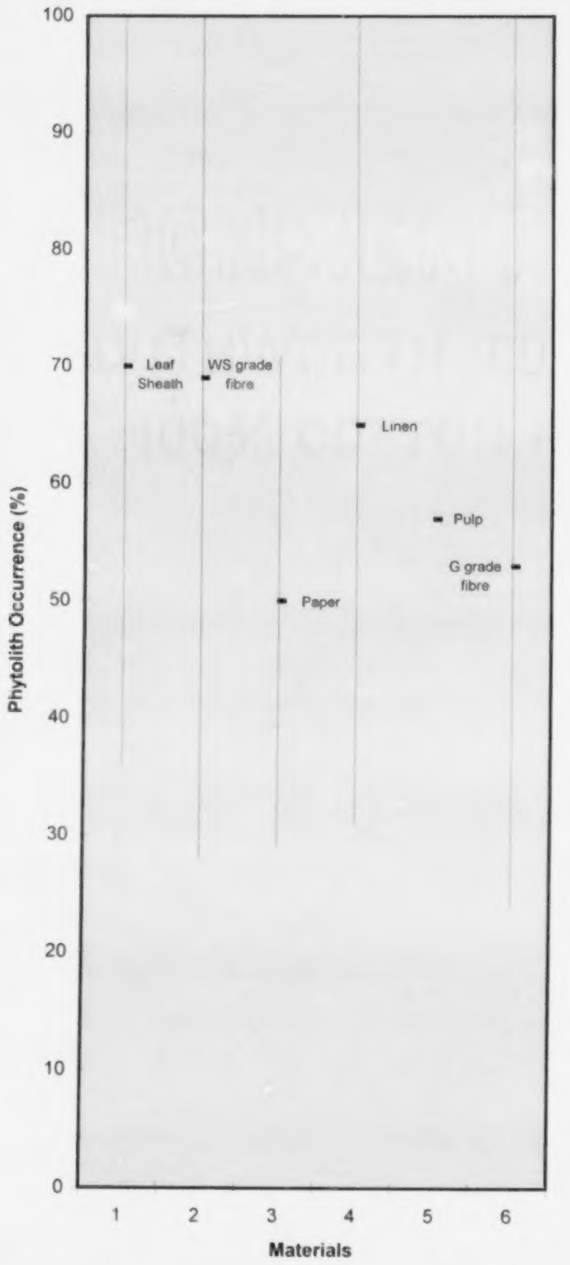

a
100 µm
b
100 µm
c
10 µm

obtainable with scanning electron microscopy. The micrograph shows multiple silica files (the fibre to the top of the image contains a long file that is not in focus) in association with the fibre bundles. Due to the nature of transmitted light, the photomicrograph shows the cross-markings on the fibre, as well as some good details about the silica units. Calcium oxalate raphide sacks or individual raphide crystals were found in 79% of the 14 leaf sheath samples and 42% of the 64 *Musa* leaf sheath, WS grade fibre, and G grade fibre samples that were examined (see data in Appendix D, Tables D1, D2, & D3). Furthermore, the location of the raphide sacks was determined to be in association with the aerenchyma tissue, at intervals, perpendicular to the vascular ribs, corroborated by the report of Franceschi and Horner (1980). Photomicrograph b), Plate 7, shows the relationship of the raphide sacs to the aerenchyma. Rhomboid crystals were found in 53% of all 98 *Musa textilis* samples (see Appendix D, Tables D1 through D6). The rhomboid crystals were originally considered to be calcium oxalate, but they responded to energy dispersive x-ray analysis as possibly being magnesium phosphate (see scanning electron micrograph c), Plate 7). They were nonetheless determined to be associated with the epidermal tissue, and were plentiful, as reported (Tomlinson, 1959). Druse crystals and crystal sand were only found in 36% of the 14 *Musa* leaf sheath samples (see Appendix D, Table D1).

Figure 9 graphically represents the observed occurrence of phytoliths in the *Musa textilis* samples. The range of percentage values is represented on the graph by the lines parallel to the y-axis, and the mean values are represented by the bar parallel to the x-axis. Examples of all the expected forms of phytoliths were observed in a mean of 70% of the 14 leaf sheath samples, 69% of the 25 WS grade fibre samples, 50% of the 14 paper samples, 65% of the 14 linen samples, 57% of the 7 pulp samples, and 53% of the 25 G grade fibre samples.

A high degree of phytolith occurrence in paper samples would relate to the presence of complete fibre bundles within the mat of fibres. However, a low overall percentage of phytoliths was observed in the paper samples, 50% of 14 samples, the

**Figure 9.** Percent observed occurrence of phytoliths in Musa textilis samples.



**Note.** Data taken from Tables C1 through C6, in Appendix C. Vertical lines enclose the range (maximum and minimum % occurrence), and the horizontal bars are mean values.

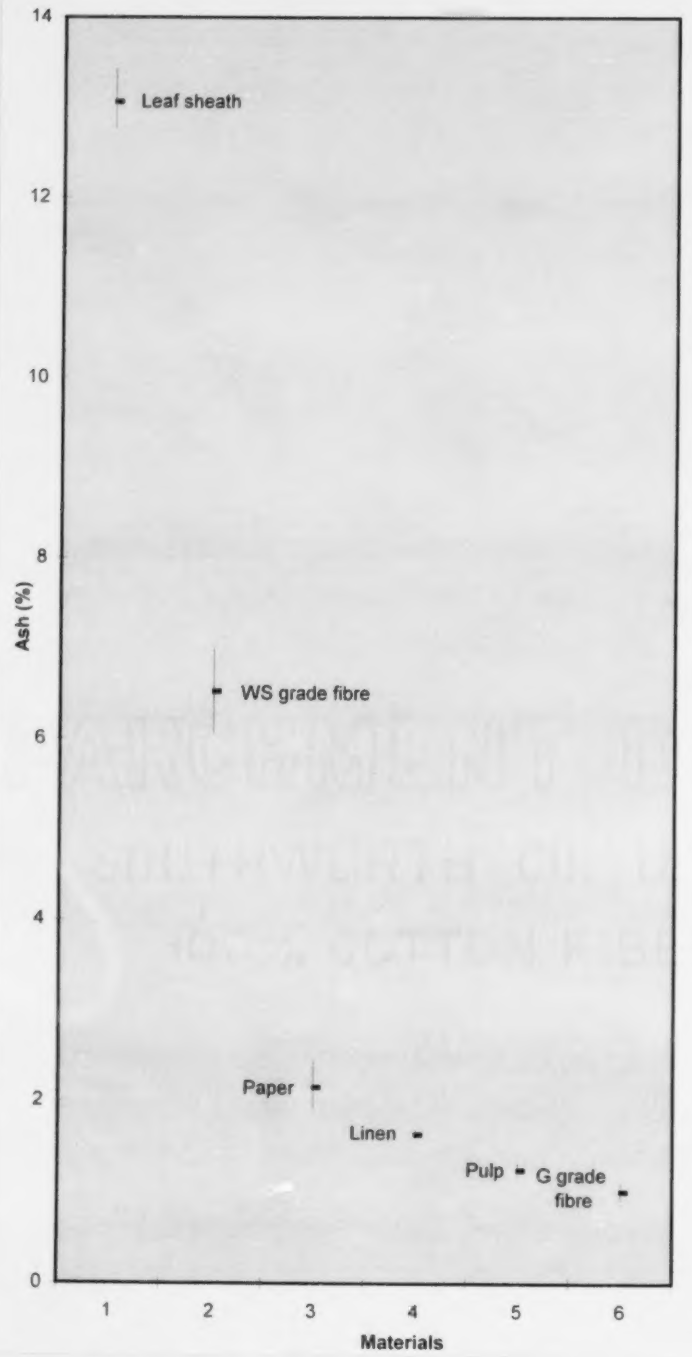
lowest amongst all the *Musa textilis* materials. The low occurrence in *Musa* paper may have been due to the difficulty of viewing the mechanically disaggregated samples. Alternately, there may simply be fewer phytoliths in *Musa* paper due to their loss the procedures involved in the paper making process. The low occurrence of phytoliths in the paper samples, relative to *Musa* linen, pulp, and G grade fibre samples, is in contrast with the *quantitative* ash content of *Musa* paper, linen, pulp, and G grade fibre samples that were thermal ashed in crucibles.

A set of qualitative results was obtained for each of the *Musa textilis* materials. The mass measurements were subsequently translated to percent, described statistically (see Table 8), and graphed (see Figure 10). The ash residue from the *Musa* paper samples had a mean mass of 2.152 %, which is higher then the percent mass of ash calculated for *Musa* linen, pulp, and G grade fibre. The higher ash content of *Musa* paper, over *Musa* linen, pulp, and G grade fibre may be a result of a sizing on the paper that may not have been removed with the deionized water washes which preceded the thermal ashing. If the greater mass of ash in the paper samples was due to sizing, and

**Table 8.** Mean and variance of the mass of ash residue from thermal ashing in crucibles, expressed as a percentage.

| *Ash %* | *Count* | *Sum* | *Mean (%)* | *Variance (%)* |
|---|---|---|---|---|
| *Musa* leaf sheath | 3 | 39.1676 | 13.056 | 0.1159 |
| *Musa* WS | 5 | 32.6027 | 6.521 | 0.1248 |
| *Musa* G | 5 | 5.0383 | 1.008 | 0.0066 |
| *Musa* "linen" | 3 | 4.9106 | 1.637 | 0.0028 |
| *Musa* Paper | 3 | 6.4546 | 2.152 | 0.0729 |
| *Musa* Pulp | 4 | 4.9628 | 1.241 | 0.0039 |

**Figure 10.** Ash (%) in Musa textilis samples thermally ashed in crucibles.



**Note:** Data taken from descriptive statistics, Table 8.

not due to the presence of phytoliths, then the observed occurrence of phytoliths (mean of 50%) would be understandable.

The occurrence of phytoliths in *Musa textilis* samples, expected as a result of the initial trials with WS grade fibres, matched the actual observed percent occurrence. In contrast, the mass of ash from thermal ashing in crucibles was different than expected. For example, I expected to measure a lower mass of ash in the *Musa* leaf sheath samples than the mass from other of the *Musa textilis* materials. I expected that there would be a greater mass of ash from the fibres which had less extraneous organic tissue, for example, G grade fibre. The reason for the discrepancy is likely a result of the high number of calcium oxalate raphide sacks, rhomboid crystals, and druse crystals in the *Musa* leaf sheath, as well as the occurrence of multiple, rather than single files of silica in association with each fibre or fibro-vascular bundle. The G grade fibre statistics offer an alternative example to the *Musa* leaf sheath example. I expected to find silica phytoliths in all the G grade fibre samples, and she did, but, unexpectedly, the % mass of ash of the G grade fibres was low. The reason for this discrepancy may be due to the greater than expected loss of most calcium oxalate crystals from G grade fibres, and the loss of some silica files during the processing of G grade fibres.

Comparison of the observed occurrence of phytoliths in the different *Musa* materials (see Figure 9), with the crucible ash content of the same materials (see Figure 10), results in an unexpected outcome. Except for the *Musa* paper results, the relative sequence of values is the same. Excluding the *Musa* paper data, the sequence from the highest to the lowest observed occurrence, or the highest to the lowest percent mass of ash is as follows: leaf sheath, WS grade fibre, linen, pulp, G grade fibre. There is a relationship between the observed occurrence of phytoliths in samples and the percent mass of ash residue from thermally ashed samples.

A limitation of the study, to focus on qualitative data, led me to dispense with quantifying the possible calcium oxalate mass in the ash, and the silica mass in the ash (see *Suggestion For Further Research*). Consequently, concluding that the differences in the percent mass of ash amongst the *Musa* samples is a result of phytolith content may be challenged. For discussion purposes one can turn to *Musa textilis* research in the Philippines, in particular, a chemical composition study by Escolano, Francia, & Semana (1977). These researchers indicate that in lower graded (courser cleaned) *Musa textilis* fibre samples, one would expect a higher mass of ash, and, in part, the higher mass is because of the high silica content. In their example, there is a 67% mean increase in mass of ash (including silica) from G grade to WS grade fibre. In contrast, there is a 133% mean increase in the % mass of silica between the grades. The difference in total mass of ash is therefore partly due to the greater silica content in the lower grade fibres. Extrapolating this information to the data represented on Figure 10 leads to the conclusion that leaf sheath samples contain the greatest mass of phytoliths, and the G grade fibres contain the least mass of phytoliths. The % occurrence of phytoliths that was observed for the various forms of *Musa* samples corroborates this conclusion (compare Figure 9, & Figure 10).

#### ***Musa*, species unknown, course grade fibre**

The *Musa* (species not *textilis*) course grade fibres were similar in quality to the *Musa textilis* WS grade fibres, and, as a consequence, the occurrence of phytoliths was similar (compare Table D2 and Table D7, in Appendix D). The total % occurrence of raphides in the *Musa textilis* samples is more similar to the *Musa* (species not *textilis*) samples when only mechanical processing, chemical digestion, and thermal ashing between glass plates at 500°C are considered, thereby bringing the total % occurrence of phytoliths in the sample sets closer together. Considering only mechanical processing, chemical digestion, and thermal ashing between glass plates at 500°C, the % occurrence for *Musa textilis* WS becomes 80%, which is closer to that observed for *Musa* (species not *textilis*) for which the three methods were used. I observed a potential subtle difference between the nature of the troughs on the *Musa textilis* silica bodies, and the nature of the troughs on the silica bodies of *Musa*, species unknown. The difference (shallower troughs, on more raised domes on *Musa*, species unknown) was not pursued, but may be at a later date. The significance of the difference may be an aid in identifiication, as is suggested by Piperno (1988), when she speaks of the difference between *Musa* and Heleconia (two *Musa*ceae) phytoliths (p. 65), and when one observes the differences in the illustrations of silica units by Tomlinson (1959) in his anatomical comparison of the *Musa*ceae.

### ***Agave cantala*** **(maguey) and** ***Agave*** **course fibre (maguey or henequen)**

Table D8, in Appendix D, summarizes the % occurrence of phytoliths in the *Agave cantala* leaf samples and *Agave* course fibre samples from the rove. Multiple files of calcium oxalate styloid crystals are associated with the fibre bundles of all *Agave cantala* leaf and *Agave* course fibre samples examined in the study. Calcium oxalate raphide sacks and individual raphide crystals were found in 50% of the 16 *Agave* course fibre samples treated in the study (see micrograph a), Plate 8). The presence of raphide crystals was not always easy to discern in the mechanically sectioned samples, the glass plate ashed samples, and the plasma ashed samples. In a few of the *Agave* course fibre samples which were mechanically sectioned, some of the finer styloids were thought possibly to be raphides. It was evident from the examination of SEM images of mechanically sectioned samples (see micrographs b) and c), Plate 8), and from two examples of raphide sacks from the chemical ashing method, that there were raphides in the *Agave* samples, but they were larger than the raphides seen in *Musa*. The raphide sacks of *Agave* contain fewer, larger raphides then the *Musa* raphide sacks.

The results of chemically ashing *Agave cantala* provide an impetus for further study (see *Suggestions For Further Research*). In every case, the organic materials of the *Agave* plant materials were digested, but the sacks that contained the styloids and raphides remained intact (see micrographs a), Plate 8, and c), Plate 9). As well as being resistant to the chemical digestion method in the study, the sacks that contain the crystals appear to provide the mechanism for aligning the crystals with the fibre bundles. Photomicrographs a) and b), Plate 9 show the plasmadesmata connections that were

PLATE 8.

**PLATE 8.**

**a)** Photomicrograph of raphide sac from *Agave* (species not determined[15]) rove fibre sample, which has been chemically digested; the containment sac has resisted chemical digestion.

**b)** Scanning electron micrograph of a calcium oxalate raphide bundle in transverse section, from a manually cut rove fibre from *Agave* (species not determined). The hexagonal perimeter of the raphides is visible in the micrograph.

**c)** Detail of micrograph b).

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[15] The species is either *Agave cantala* (maguay), or *Agave fourcroydes* (henequen), as is discussed in the *Materials and Methods* section.

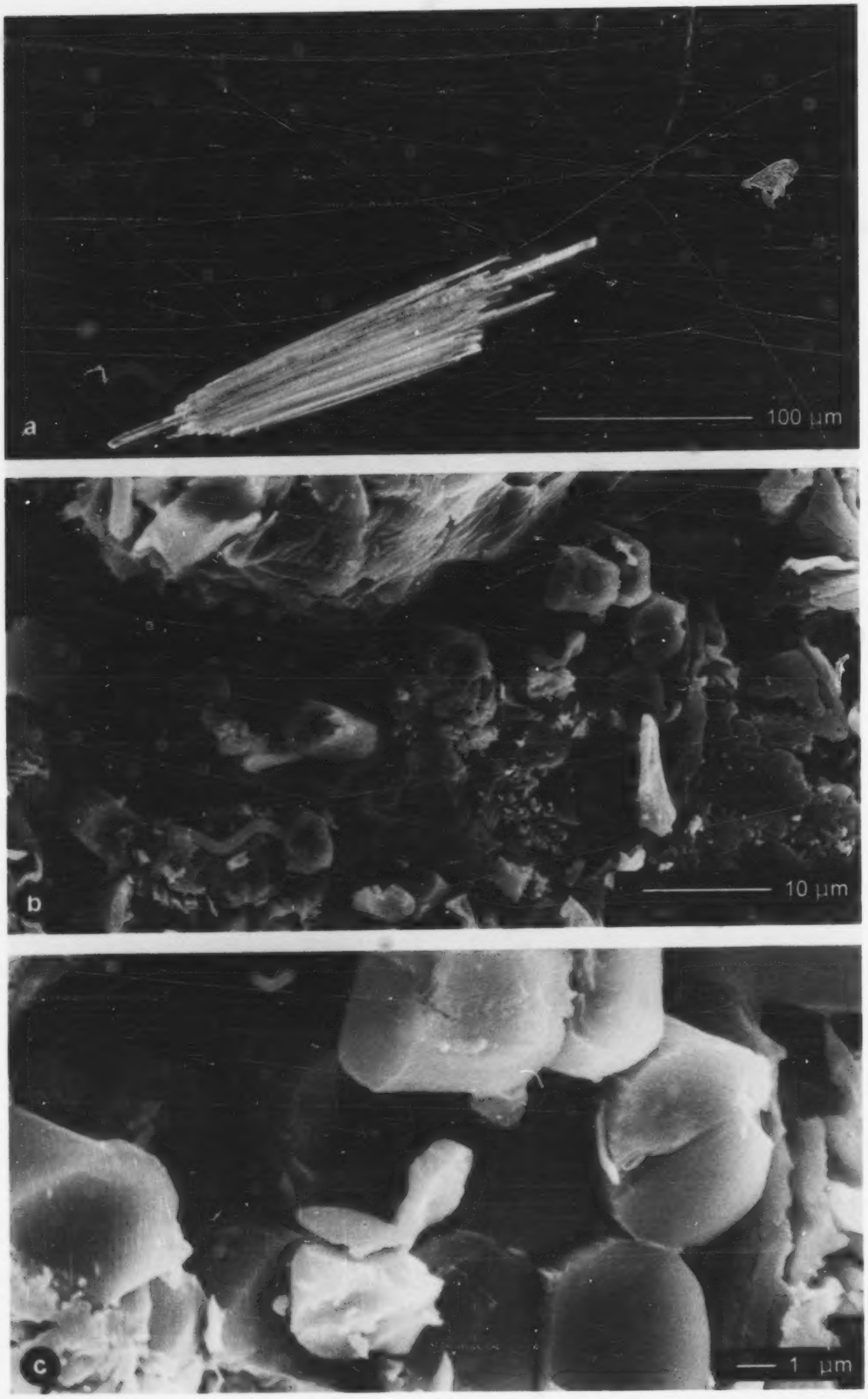
a
100 µm
b
10 µm
c
1 µm

**PLATE 9.**

**PLATE 9.**

**a)** Photomicrograph of *Agave* (species not determined[16]) rove fibre sample, which has been manually processed, showing the alignment and attachment of calcium oxalate styloid crystals to the fibre bundle.

**b)** Detail of a), showing the plasmodesmata connections more clearly. This micrograph also shows that the styloid containment sac, or lithocyst is longer than the styloid, perhaps allowing for further longitudinal growth of the styloid.

**c)** Photomicrograph of a pair of styloids in one lithocyst, from *Agave* (species not determined) rove fibre sample, which has been chemically digested; the lithocyst has resisted chemical digestion.

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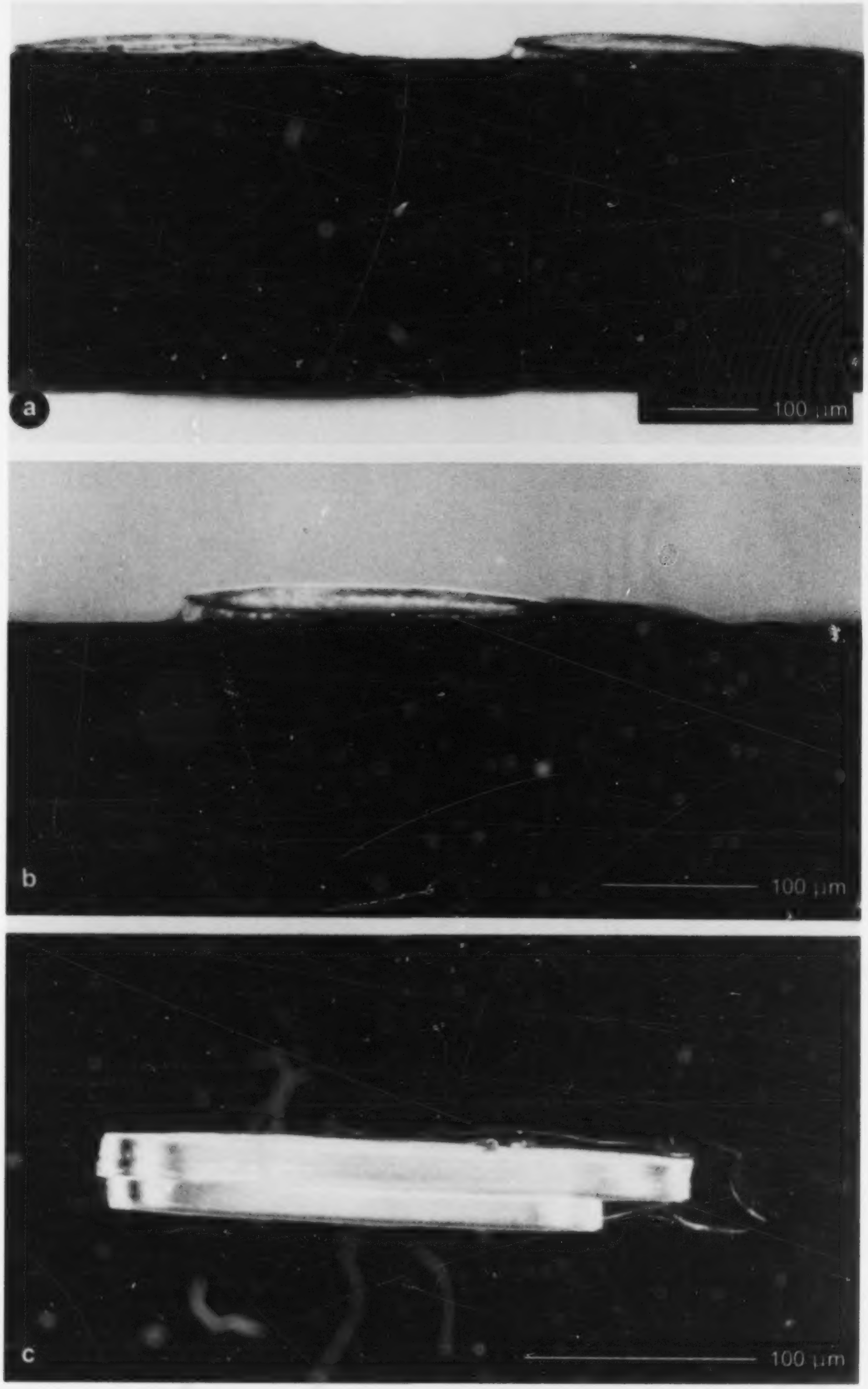
[16] The species is either *Agave cantala* (maguay), or *Agave fourcroydes* (henequen), as is discussed in the *Materials and Methods* section.


a
100 µm
b
100 µm
c
100 µm

observed occurring between the fibres and the crystal sacks, or lithocysts, of many samples. The sacks in some cases contained single crystals, but in some cases they contained pairs of crystals that were not often equivalent in scale; in some cases 50 μm crystals were paired with 350 μm crystals (see photomicrograph c), Plate 9).

The clean crystals from the chemical digestion method were easily viewed with all the imaging means of the study. Under polarized light the crystals displayed interference colours that ranged from straw yellow to blue depending on the thickness of the crystal being viewed. When larger crystals were observed in plan view, the interference colours appeared as bands, radiating from the thicker middle of the crystal, along the length, to the thin ends of the crystal. When viewed in elevation, the crystals were one colour overall. The difference in the appearance of the interference colours from the plan to elevation views supports the proposal that the crystals are formed in the way they are represented in Figure 3 and as they are shown in the two scanning electron micrographs in Plate 10. The forms in which the crystals appear after being glass plate ashed can then be seen as distortions of the forms seen following mechanical sectioning or chemical digestion. The glass plate ash *Agave* styloid crystals are coated with tar, bent laterally and longitudinally, and broken -- the "small black bananas" reported by Catling and Grayson (1982, p. 56).

The relationship of the styloid crystals to the *Agave* fibre bundles was revealed as being via the sacks that contain the crystals, and the raphide sacks were determined to be associated with the ground parenchyma. Photomicrographs b) and c), Plate 8, illustrate a portion of a raphide sack that has been sectioned transversely, within a section of ground, epidermal, and fibre tissues. The raphide crystals have a variety of perimeter forms, depending on their positioning within the bundle. Raphides to the exterior of the bundle are closer to cylindrical, with one or two tangential sides. The raphides nearer the centre of the bundle have perimeters that are closer to hexagonal. This same hexagonal form was observed by Wattendorff (1976b). (The forms of the raphides indicate a possible reason for the bundle remaining like a close packed whole, rather then like a

# PLATE 10.

**PLATE 10.**

**Two scanning electron micrographs of calcium oxalate crystals from manually sectioned *Agave* (species not determined[17]) rove fibre.**

**a)** Plan and elevation views of a pair of calcium oxalate styloid crystals.

**b)** Transverse section of a calcium oxalate styloid crystal adjacent to a fibre bundle. The rectangular surface suggests that the section is from a location nearer one end of the crystal.

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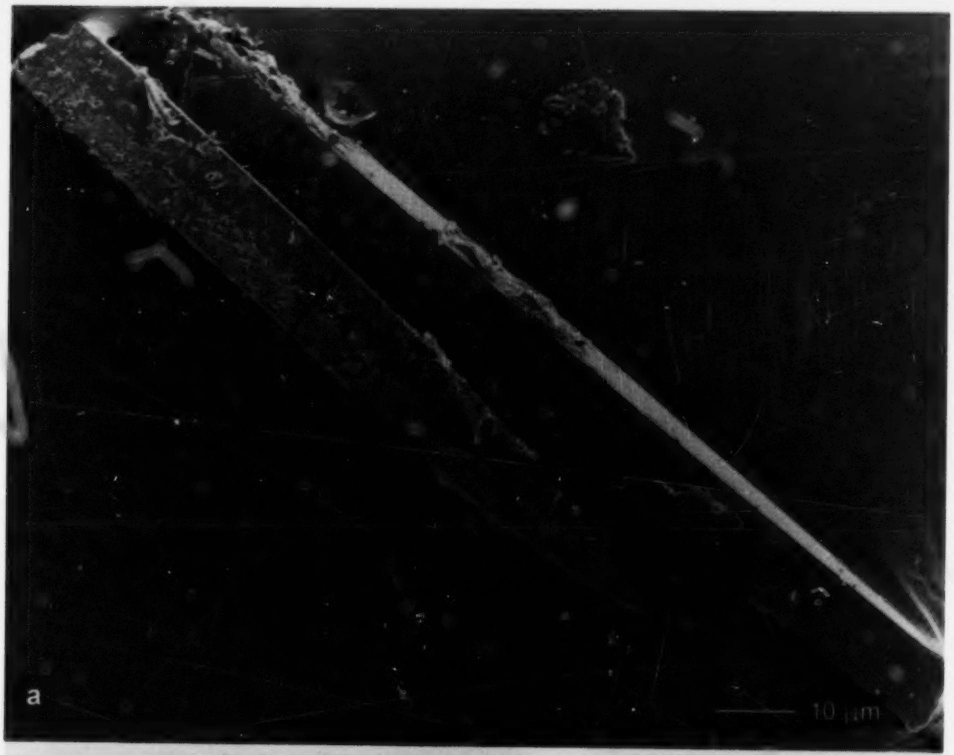
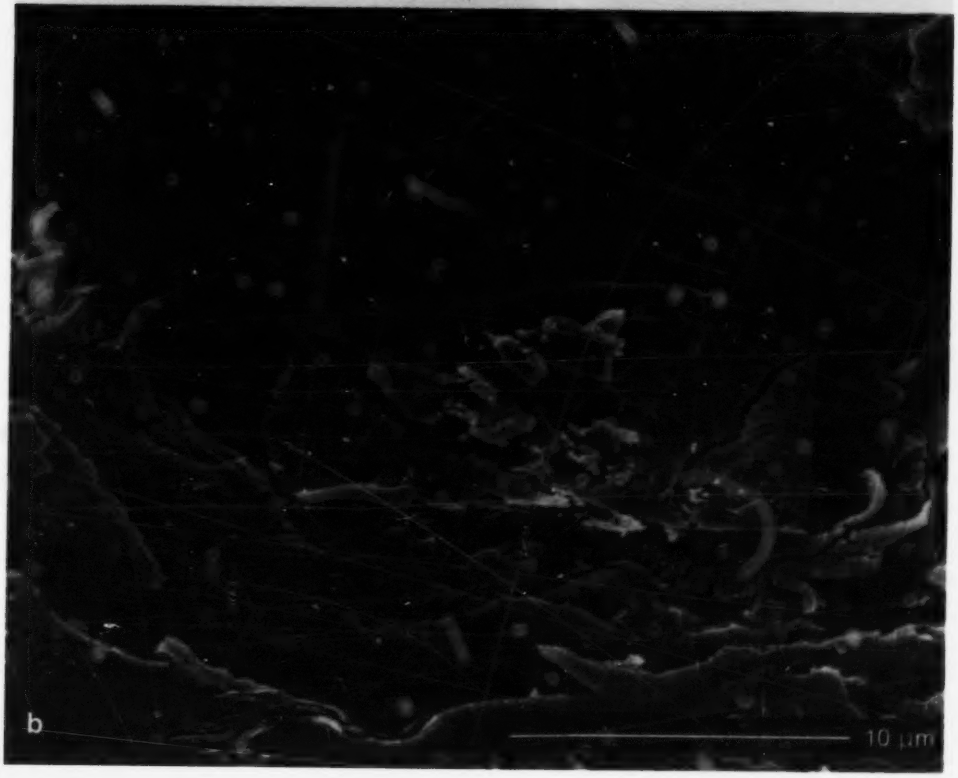
[17] The species is either *Agave cantala* (maguay), or *Agave fourcroydes* (henequen), as is discussed in the *Materials and Methods* section.


a
10 µm


b
10 µm

loose sack of needles. There was no transverse section made of a raphide sack from *Musa textilis*, but the fact that the *Musa* raphide bundles were determined, with the vertical scan process on CLSM, to be as deep as they were wide, suggests that, the raphides in *Musa* may not be precisely cylindrical.

### *Agave* (*cantala*, maguey or *fourcroydes*, henequen) rope

Table D9 charts the % occurrence of phytoliths in *Agave rope* samples (see Appendix D). Calcium oxalate styloid crystals are associated with the fibre bundles of all 10 of the *Agave* rope samples examined in the study. The majority of the crystals were in place as individuals, although occasionally there was an indication that the crystals were in files. No calcium oxalate raphide sacks or single crystals were found in any of the *Agave* rope samples in the study. No raphides were found, even though raphides are expected in less processed materials. Although styloids can be expected in 100% of the samples, the quantities vary greatly, and often the styloids are broken. The residue from glass plate thermal ashing provides styloid crystals that are contaminated with tar and bent, in the same way they were observed for *Agave cantala* and *Agave* course fibre samples.

### *Boehmeria nivea* (L.) Gaud. (ramie)

Table D10 summarizes the % occurrence of phytoliths observed on fibre ultimates of *Boehmeria nivea* (see Appendix D). Calcium oxalate druse crystals were observed in 35% of the 17 samples examined (each sample contained 10 fibre ultimates); a rhomboid crystal was observed on one of the samples; and crystal sand was reported for 50% of the samples. Most of the observations were of degraded or fragmented druse crystals. The single occurrence of the rhomboid crystal may be inaccurate. Rhomboids are not reported by Catling and Grayson (1982) as being characteristic to the species, and a single observation may indicate that it is possibly an artifact. The single rhomboid was not subject to EDXA, and, therefore, it is not necessarily calcium oxalate. The occurrence of crystal sand may be an artifact of degraded druse crystals, or of other debris. The chemically ashed *Boehmeria* ultimates may have lost the loose and disaggregated fragments of druse crystals. In general, the highly refined nature of the fibre ultimates makes it unlikely that calcium oxalate druse crystals would be found, although Catling & Grayson (1982) report their presence in *Boehmeria nivea*.

### ***Corchorus capsularis*** **L. (jute)**

In accordance with reported occurrences (Catling & Grayson, 1982; Jarman & Kirby, 1955), individual crystals and files of calcium oxalate rhomboid crystals were found in 100% of the 17 samples examined (see Appendix D, Table D11). Although an occurrence of individual calcium oxalate druse crystals is reportedly possible according to Catling and Grayson, none were observed in the study. In their report, Jarman and Kirby also found the occurrence of druse crystals to be low or non existent in stripped fibres. The rove used in the current study yielded fibres that were cleaned of most ground parenchyma, where the druse crystals are reportedly located. In contrast, files of rhomboids were abundant in all samples treated by a variety of methods. The different views of the crystals that were allowed by the different methods enabled me to begin to define the shape of the crystals as truncated pyramids, with the base of the pyramid towards the perimeter of the fibre bundles. The SEM work indicated the flat, square upper surface, and the chemical digestion and thermal ash resides provided views of the axehead or pyramid shape. The study yielded insufficient data to define the association of the crystals with the fibre bundles, however, micrographs a) and b), Plate 11, begin to suggest the relationship. The crystals appear to be enclosed in a file of organic encasements that are to the extremity of, but within the perimeter of, the fibre bundles. The same placement was observed for the styloid crystals of *Agave*, and the silica bodies of *Musa*. In general, the results of the current study suggest that the presence of files of rhomboid crystals, or former crystal encasements on *Corchorus capsularis* fibre bundles provides a characteristic that appears to be definitive and to persist through degradation.

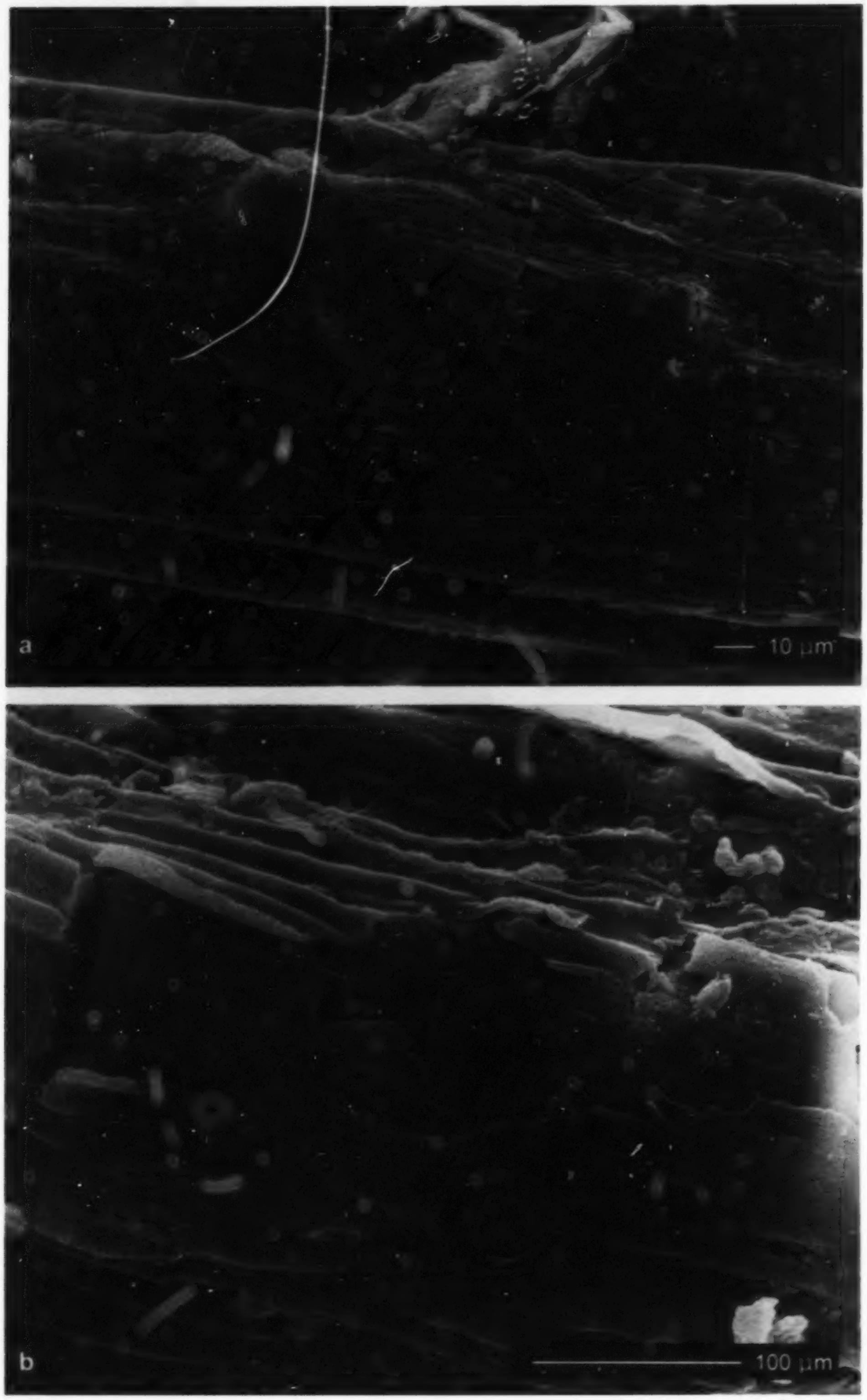
PLATE 11.

## PLATE 11.

**Two scanning electron micrographs of a *Corchorus capsularis L.* (jute) rove fibre, which has been manually processed.**

**a)** Fibre bundle with three calcium oxalate rhomboid crystals in position near a node.

**b)** Detail of calcium oxalate rhomboid crystals showing the organic material in which they are encased.


a
10 µm
b
100 µm

### ***Cannabis sativa* L. (hemp)**

Table D12 summarizes the % occurrence of phytoliths in five *Cannabis sativa* stem samples (from one stem) and 16 *Cannabis sativa* summer cloth samples (see Appendix D). Trichomes were found in all of the *Cannabis sativa* stem samples, and silicified trichome bases were observed in 37.5% of the summer cloth samples. Except in one possible case, the trichome bases were difficult to discern in the thermal ash residue, but they were readily discernible in the residue resulting from chemical digestion. Short files of calcium oxalate druse crystals or individual druse crystals were observed in all 5 of the stem samples and in 81.3% of the 16 summer cloth samples. The files of calcium oxalate druse crystals are associated with the phloem fibre bundles, and the individual druse crystals are associated with the ground parenchyma adjacent the phloem fibre bundles, or within the pith parenchyma (see Plate 12). These findings are in accordance with published results (Dayanandan & Kaufman, 1976; Catling & Grayson, 1982).

In transverse sections of the stems the druse crystals appear scattered around the phloem fibre bundles, and scattered within the pith parenchyma. However, observing a radial section of a stem, one can discern that the druse crystals, appearing in transverse sections to be single crystals near the phloem fibre bundles, are actually individuals within a file that runs parallel to a fibre bundle. Reviewing the drawing of *Cannabis sativa*, as well as the drawings of *Boehmeria nivea* and *Corchorus*, in Catling and Grayson (1982, Figures 5, 6, 9, 10, 11, and 12), one becomes aware that the association of the crystal files with the fibre bundles is consistent with the findings obtained for *Musa textilis* and the *Agave*s that were studied. For *Cannabis sativa*, the files are not always composed of druse crystals only. In one instance, a file of druse crystals contained a diamond rhomboid (see Plate 12 ), and in some instances fragments of crystals were observed. The crystal sand observations reported for the stem and cloth samples may be the result of disaggregated druse crystals; with chemical digestion and thermal ashing, the sand (or fragments of druse) were absent or difficult to discern. Mechanical sectioning may cause the druse crystals to be displaced or shattered, and

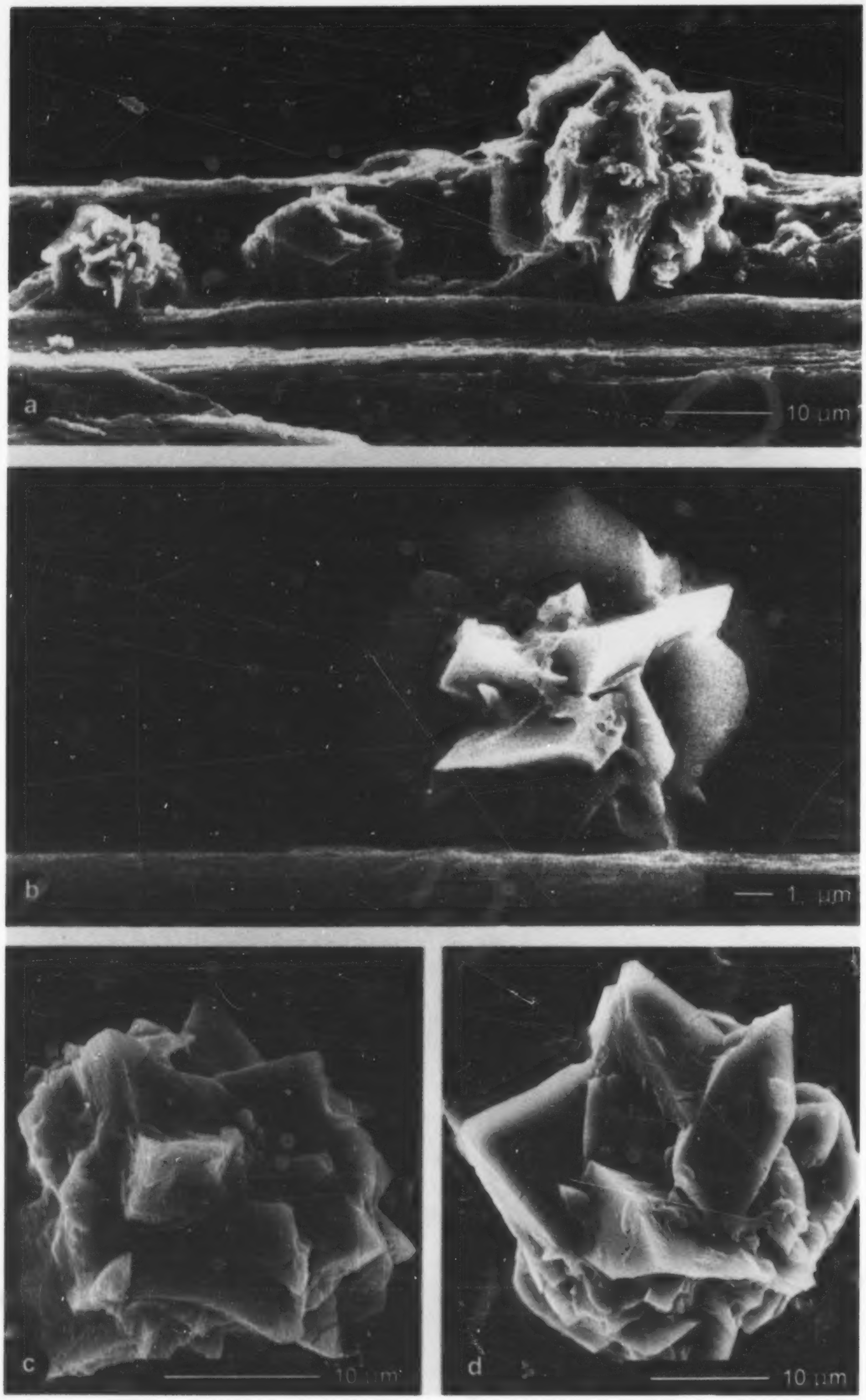
**PLATE 12.**

**PLATE 12.**

**Four scanning electron micrographs of calcium oxalate crystals from *Cannabis sativa* L. (hemp).**

**a)** Three calcium oxalate crystals in place on a fibre bundle. The druse crystals can vary in dimension, and, occasionally, there are crystals that are closer to rhombohedral form. The rhomboids tend not to have clean faces; they tend to have fragments attached to their surfaces.

**b), c), and d)** Examples of single calcium oxalate crystals.


a
10 µm
b
1 µm
c
10 µm
d
10 µm

the short files that are observed may be longer files that have been disrupted.

### *Agave sisalana* (sisal)

In accordance with reported occurrences (Catling & Grayson, 1982), individual calcium oxalate styloid crystals and short files of calcium oxalate styloid crystals were observed in all five of the ash residues from *Agave sisalana* rope samples. Glass plate thermal ash treatments of the *Agave sisalana* samples were all that was done for the study, simply to determine the presence or lack of styloid crystals. The resulting residue contained tarred, bent, and broken calcium oxalate styloid crystals in parallel association with the fibre bundles. The quantity and form were consistent with the crystals found in the rope samples of *Agave*, species not determined.

# CHAPTER 5: SUMMARY, CONCLUSIONS, AND SUGGESTIONS FOR FURTHER RESEARCH

## Introduction

The overall purpose of the research was to determine if phytolith analysis is a viable method of cellulosic materials identification for use by conservators. An underlying purpose of the research was to contribute to phytolith reference collections. The research began with an evaluation of seven methods of phytolith distillation and concluded with an examination of the phytolith content of a set of cellulosic materials. This chapter summarizes the evaluations of the methods used and summarizes the phytolith data that were collected. Conclusions are also made about the use of phytolith analysis by conservators, and suggests further research that will be necessary before phytolith analysis can be incorporated into current cellulosic materials identification protocols.

## Summary And Conclusions Of The Seven Methods Of Phytolith Distillation

Table 4 provides a summary of the seven methods of phytolith distillation that were evaluated in the study. In my experience, the mechanical processing method and the oxygen plasma ashing method each provided good overall results. Mechanical processing is safe, is relatively inexpensive, uses equipment that is easy to obtain and operate, and yields valuable data. However, mechanical processing can be time consuming and requires skill, and therefore a more expedient method may need to be chosen. The main advantage of the oxygen plasma ashing method is the distinctive nature of the degraded fibres (see Plate E1 in Appendix E), and the retention of phytolith data. The drawbacks of oxygen plasma ashing are the relative inaccessibility of the equipment, the length of time required to ash specimens, and the fragility of the ash residue. Before plasma ashing can become a viable technique, further research will be needed to determine the best plasma for ashing, and a way of minimizing disturbances to the ash residue. The method of thermal ashing between glass plates at 500°C can be desirable if it's limitations (phytolith data disruption and potential thermal distortions ) are acknowledged. Thermal ashing is safe, inexpensive, quick, and uses equipment that is relatively accessible and relatively easy to operate. Chemical digestion is also relatively quick (mostly because multiple specimens can be processed at once), but has a health risk, and is expensive. Another caution is that chemical digestion, which only provides a summary of information, that is separate from the context of the plant anatomy, should not be used alone when reference collections are being built. Reference collections require the phytoliths to be represented in the context of plant anatomy.

I have concluded that, for the creation of reference collections, the most expedient way to proceed was to make a quick thermal ashing between glass plates at 500°C, and commence chemical digestion, and, if feasible, plasma ashing. Mechanical processing can proceed concurrently with the thermal and oxygen plasma ashing and chemical digestion. With the sequence of methods, requiring multiple samples, a clear picture of the various characteristics of the cellulosic material would be provided. The recording of collected data should follow procedures described in *Materials and Methods*.

When working with degraded materials, I concluded that mechanical processing, which is already a part of most identification protocols, be conducted first. If there is material that can be spared from the artifact being examined, the next step would be to proceed with thermal ashing between glass plates at 500°C, chemical digestion, and oxygen plasma ashing. If the material being analyzed is degraded beyond visual recognition (in cases the material being examined may already be thermally ashed or chemically degraded) and will not react to chemicals in a predictable way, then I would conclude that mechanical processing be followed by chemical digestion, and, possibly thermal and oxygen plasma ashing. For almost all degraded artifacts, the decision to perform an analysis would be on a case by case basis, beginning with a consideration of provenance, and incorporating the principles and practices of conservation. By establishing provenance, the fibre identification may be evident without scientific analysis. By incorporating the principles and practices of conservation, the removal of appropriate volumes of fibres for scientific examination, and the appropriate handling and storage of sensitive objects will be maintained.

### Summary And Conclusions Of The Phytolith Data Collected

Two significant findings emerged from the study of the phytolith data. The first is the fact that phytoliths of *Musa*, *Agave*, *Cannabis*, *Corchorus*, and *Boehmeria* tend to be not only aligned with the fibre and fibro-vascular bundles, but they also appear to be connected to the bundles by their containment cells, or lithocysts. This association requires further study, and it provides impetus for further development of phytolith systematics within the field of textile conservation. If the association is consistent within all species of plants that yield textile fibres, then there is reason to continue to pursue the development of phytolith keys for those species. A limitation of the finding might be the loss of the phytolith/fibre association during processing of the plant to harvest the fibres, or the manipulation of the fibres to make the cloth. The second finding addresses this limitation.

The second finding is the fact that although more highly processed materials tend to exhibit fewer phytoliths, they still tend to contain, at least, individual phytoliths. During processing, a percentage of the phytolith data is maintained. This was the case for the *Musa textilis* (abaca, or Manila hemp) paper and pulp samples, the *Agave* (maguay or henequen, and sisal) rope samples, the *Corchorus capsularis* (jute) rove fibres, and the *Cannabis sativa* (hemp) fabric samples. This observation is significant for cellulosic materials with species-specific, or, in some circumstances, genus-specific phytoliths (*Musa textilis*, *Agave*, and *Corchorus*), and also for species that contain phytoliths found amongst many species (redundant). In instances where the materials are thoroughly stripped, and cleaned, or, perhaps, cottonized (partially degraded chemically, to yield fibre ultimates), one would not expect to find any phytoliths, but they may sometimes be there. The occurrence of specific phytoliths provides direct assistance with the identification process, and the occurrence of non-specific phytoliths can assist with the identification by narrowing the possibilities.

Beyond these significant results, this portion of the research has provided data that will contribute to phytolith reference collections. Most of the observations have been made in the botanical or textile science literature, but not incorporated into phytolith systematics. In particular, the research determined that the silica bodies of *Musa* are files of non-uniform units, in association with the fibre or fibro-vascular bundles. I also determined that the crystal rhomboid forms of *Musa* originate from one basic form. Also of significance was the finding that the raphide sacks in *Musa* are located in the aerenchyma tissue, and that there is a difference between the raphide sacks of *Agave* and *Musa*. Lastly, the research indicates the potential that files of calcium oxalate styloid, druse, and rhomboid crystals may be longer than seen in longitudinal view (shorter files may result from displacement during sectioning), and that the calcium oxalate crystals are not randomly oriented, as they appear in transverse view.

## Phytolith Analysis As A Tool For Conservators

Some conservators are aware that cellulosic materials may contain microscopic inorganic materials, such as silica and calcium compounds (Florian, 1981; Körber-Grohne, 1991; Schaffer, 1981); however, phytolith analysis, as a systematic approach, has not been formalized for textile conservation work. Fibres from artifacts are usually identifiable by less intrusive means than are necessary with phytolith analysis. In contrast, there are instances when highly degraded cellulosic materials, such as chemically or thermally ashed textiles, require other than conventional identification techniques. Additionally, when a set of cellulosic materials is too similar in character to be differentiated by conventional means, other techniques of identification are necessary. The results of the study have indicated that phytolith analysis is a technique that could be developed further for material culture conservators. The challenge at this time is the lack of identification keys, or reference collections. This study has provided data that could be incorporated into existing keys, but more work will need to be done to develop a thorough key for other species or genera of cellulosic materials. When plant materials are analyzed the researcher is well advised to follow the key development system presented by Brown (1984), with the addition of a seventh step which shows the phytolith assemblages within a graphic three dimensional representation of the plant (Parry & Smithson, 1962). An additional challenge for conservators may be the skill required to perform some of the phytolith analysis methods, such as mechanical sectioning and microscopy, or chemical digestion and plasma ashing.

Catling and Grayson (1982) began the process of developing a key for the phytoliths found in plants that yield fibres for textiles when they brought together "crystal" data in their book, Identification of vegetable fibres. They did not refer to the crystals that they had observed as phytoliths, and their observations are sketchy in cases, but they have provided a good foundation from which to work. The updating of Catling and Grayson's work that is necessary includes a thorough analysis of the plant organs and tissues that yield fibres, following Brown's system (1984). Their work also needs a clarification of the *Agave* fibre/crystal relationship; in fact, one of the three fibro-vascular bundles in the micrograph of *Agave sisalana* in their book (p. 53) does show the "boxed" area where two phytoliths have been displaced during sectioning. There needs to be an illustration of the Sunn hemp crystals that are reported, but not indicated on the micrograph. And, lastly, a new edition would need to incorporate further discussions of the significance of the fibre/phytolith associations. In general, the association of phytoliths with plant fibres indicates that phytolith analysis could evolve to be an appropriate identification tool for use by conservators.

**Suggestions For Further Research**

In addition to determining that phytolith analysis is a worthwhile pursuit to aid in the identification of cellulosic materials, other data arose from the research that indicated the need for further studies. In the area of botanical research, some work could be done to test and evaluate the reason for the phytolith/fibre sclereid association, to evaluate the role of phytoliths in either structural or intrusive growth roles, and to determine why the area of the fibre sclereids is the phytolith deposition location in plants that yield fibres.

The quantification of phytolith content in cellulosic materials used in textiles could be pursued. The total mass of ash remaining after ashing samples in crucibles in the current study was determined, but the silica versus non-silica inorganic content was not quantified. Lanning and Eleuterius (1991) presented a method for determining, by difference, the non-silica inorganic content and, then, the silica content of ash. The steps include a hydrochloric acid wash of the ash, followed by quantification, and, then a hydrofluoric acid wash, followed by quantification. In the future the process could be conducted on the residues saved from the six sets of crucible ash trials in the current study.

Phytoliths from a genus that contains many species that yield fibres for textiles could be compared. For instance, the current study was limited in its ability to establish the species of *Agave* that yielded the rove of course fibres, or the rope that bound the rove together. The samples of *Agave sisalana* did not provide enough data to make assumptions about the differences between *Agave sisalana* or other *Agave*. Perhaps a study that focused on the similarities and differences between the styloids and raphides of various species of *Agave* would assist in the differentiation of species. The areas to focus on would include crystal size, crystal pairing, raphide sack contents, the lithocysts that contain the raphides and styloids, the number and placement of styloid files associated with each fibre bundle, and the overall quantification and occurrence of the crystals in the samples studied. The information would need to be paired with cultural context information, including processing, distributing, and consuming patterns (Sibley and Jakes, 1989).

Lastly, the indication that fibres vary in their morphology when degraded, as outlined in Plate E1 (Appendix E), could be further researched and possibly complement the protocol of Marshall (1992). There are variations in the way different materials appear when chemically digested, thermally ashed (see Table E1 in Appendix E), or oxygen plasma ashed. These variations could aid in the identification process if they were thoroughly researched and documented.

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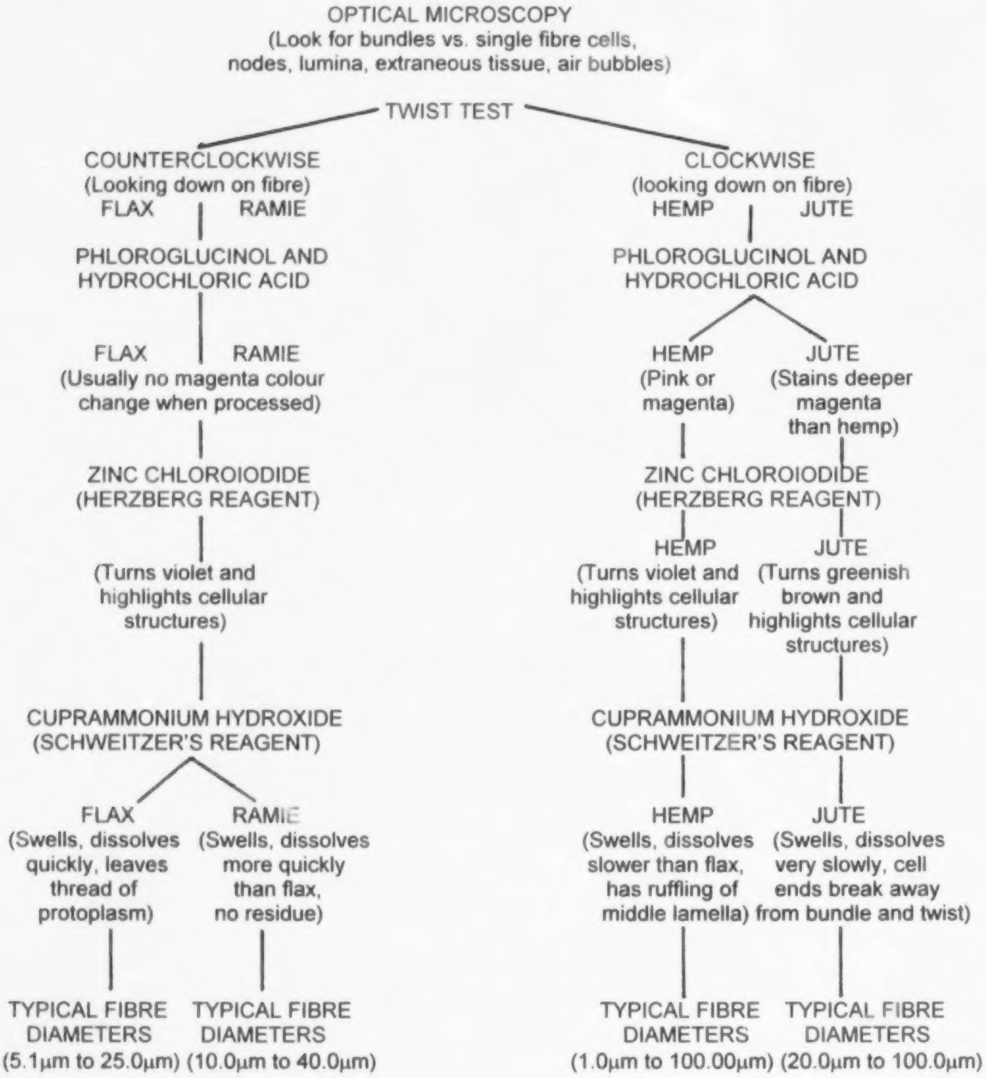
# APPENDICIES

# APPENDIX A:

## Marshall Protocol

## TEST PROTOCOL AND SUMMARY OF OBSERVATIONS


OPTICAL MICROSCOPY
(Look for bundles vs. single fibre cells, nodes, lumina, extraneous tissue, air bubbles)
TWIST TEST
COUNTERCLOCKWISE
(Looking down on fibre)
FLAX
RAMIE
CLOCKWISE
(looking down on fibre)
HEMP
JUTE
PHLOROGLUCINOL AND HYDROCHLORIC ACID
PHLOROGLUCINOL AND HYDROCHLORIC ACID
FLAX
RAMIE
(Usually no magenta colour change when processed)
HEMP
(Pink or magenta)
JUTE
(Stains deeper magenta than hemp)
ZINC CHLOROIODIDE (HERZBERG REAGENT)
(Turns violet and highlights cellular structures)
ZINC CHLOROIODIDE (HERZBERG REAGENT)
HEMP
(Turns violet and highlights cellular structures)
JUTE
(Turns greenish brown and highlights cellular structures)
CUPRAMMONIUM HYDROXIDE (SCHWEITZER'S REAGENT)
FLAX
(Swells, dissolves quickly, leaves thread of protoplasm)
RAMIE
(Swells, dissolves more quickly than flax, no residue)
CUPRAMMONIUM HYDROXIDE (SCHWEITZER'S REAGENT)
HEMP
(Swells, dissolves slower than flax, has ruffling of middle lamella)
JUTE
(Swells, dissolves very slowly, cell ends break away from bundle and twist)
TYPICAL FIBRE DIAMETERS (5.1μm to 25.0μm)
TYPICAL FIBRE DIAMETERS (10.0μm to 40.0μm)
TYPICAL FIBRE DIAMETERS (1.0μm to 100.00μm)
TYPICAL FIBRE DIAMETERS (20.0μm to 100.0μm)

**Note.** From The identification of flax, hemp, jute and ramie in textile artifacts, (p. 136) by J. Marshall, 1992, Edmonton: University of Alberta, unpublished masters thesis. Copyright 1982 by J. Marshall. Reprinted by permission.

# APPENDIX B:

## Materials And Methods Record Sheets

**Table B1.** Assignment of data record numbers to samples.

DATA SHEETS

| *MATERIALS* | | *SAMPLES* |
|---|---|---|
| *MUSA TEXTILIS* | leaf sheath | 0001 - 0099 |
| | residual fibre (WS) | 0100 - 0199 |
| | high grade fibre (G) | 0200 - 0249 |
| | products (paper) | 0250 - 0269 |
| | products (pulp) | 0270 - 0299 |
| | products (linen) | 0300 - 0329 |
| | | 0330 - 0349 |
| *AGAVE CANTALA* | leaf tissue | 0350 - 0399 |
| | course fibre | 0400 - 0429 |
| | | 0430 - 0449 |
| | product (rope) | 0450 - 0469 |
| *BOEHMERIA NIVEA* | stems | 0470 - 0499 |
| | fine fibre (roving) | 0500 - 0549 |
| | | 0550 - 0569 |
| *CORCHORUS CAPSULARIS* | stems | 0570 - 0599 |
| | fibre | 0600 - 0629 |
| | | 0630 - 0649 |
| | | 0650 - 0699 |
| *CANNABIS SATIVA* | stems | 0700 - 0749 |
| | product (rope or sacking) | 0750 - 0799 |
| | product (summer cloth) | 0800 - 0829 |
| | product (linen) | 0830 - 0859 |
| | | 0860 - 0899 |
| | | 0900 - 0949 |
| | | 0950 - 0999 |
| MISC: *MUSA TEXTILIS* | miscellaneous | 1000 - 1049 |
| *AGAVE CANTALA* | miscellaneous | 1050 - 1099 |
| *MUSA (NOT TEXTILIS)* | miscellaneous | 1100 - 1124 |
| *BOEHMERIA NIVEA* | miscellaneous | 1125 - 1149 |
| *CORCHORUS CAPSULARIS* | miscellaneous | 1150 - 1199 |
| *CANNABIS SATIVA* | miscellaneous | 1200 - 1249 |
| *AGAVE SISALANA* | product (rope) | 1250 - 1299 |
| *SPINACIA OLERACEA* | leaf | 1300 - 1350 |

**Table B2.** Mechanical Processing Check List.

## *Mechanical Processing Check List*

* Collection Context
* Sample on Page
* Sample Gross Descrip.
  - dimensions
  - colour
  - texture
  - weave
  - ply
  - twist
* Condition
  - Overall Class
    - excellent
    - very good
    - good
    - fair
    - poor
  - Deterioration
  - Stains, soil, discoloration
  - Previous treatment or Repair
* Context of Plant Anatomy
* Context of Plant Metabolism
* Processing Methods
* Sample Size relative to Plant
* Longitudinal (tangential, radial) sections
  - mountant
  - magnification
  - dimensions
  - tissue types
  - cell types
  - fibre cell ends
  - fibre cell markings (cross, dislocation)
  - fibre cell wall
  - fibre cell lumen
  - crystals
  - debris other than cellular
* Transverse section
  - mountant
  - magnification
  - dimensions
  - tissue types
  - cell types
  - fibre cell wall
  - fibre cell lumen
  - cell-cell interactions
  - cell-cell spatial relationships
  - crystals
  - debris other than cellular
* Cleared Longitudinal Sections
* Cleared Transverse Sections
* Polarizing Light Response
* Birefringence
* Burn Characteristics
  - near flame
  - ignition
  - in flame
  - flame colour
  - ash colour
  - ash texture

**Table B3.** Mechanical Processing Record Table.

### *Mechanical Processing Record Table*

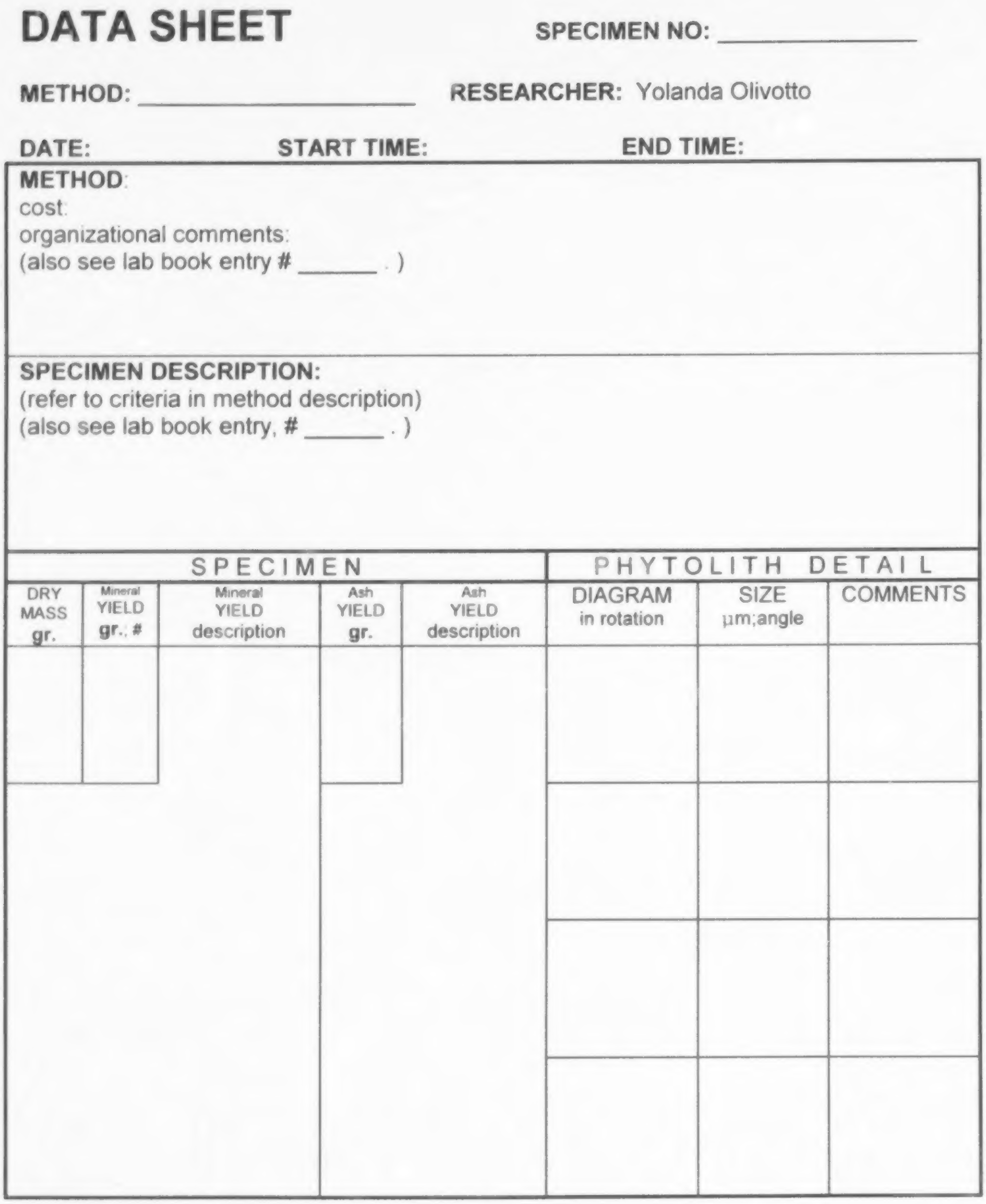
| Criteria descriptions a to n at end of table | | a | b | c | d | e | f | g | h | i | j | k | l | m | n | OTHER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Musa*** (leaf sheath) | **001a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Musa.*** (W5) | **100a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| ***Musa*** (G) | **230a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Musa*** (paper) | **250a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Musa*** (pulp) | **270a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Musa*** (linen) | **300a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Musa*** (not t.) | **1100a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Agav.*** (leaf) | **350** | | | | | | | | | | | | | | | |
| ***Agav.*** (course fibre) | **400a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |

| | | a | b | c | d | e | f | g | h | i | j | k | l | m | n | OTHER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Agav.*** (rope) | **450a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Boeh.*** (roving) | **500a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Corc.*** (fibre) | **600a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Cann.*** (stems) | **700a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |
| ***Cann.*** (summer cloth) | **800a** | | | | | | | | | | | | | | | |
| | b | | | | | | | | | | | | | | | |
| | c | | | | | | | | | | | | | | | |
| | d | | | | | | | | | | | | | | | |
| | e | | | | | | | | | | | | | | | |

*Agav.* = *Agave cantala*, or **Maguey; also *Agave sisalana***
*Boeh.* = *Boehmeria nivea* (L.) Gaud, or **Ramie;**
*Cann.* = *Cannabis sativa* L., or **Hemp**;
*Corc.* = *Corchorus capsularis* L., or **Jute;**
*Musa* = *Musa textilis* Née, or **Abaca**, or **Manila Hemp.**

a = Collection context.
b = Saved sample.
c = Sample description.
d = Condition.
e = Context of plant anatomy.
f = Context of plant metabolism.
g = Processing methods.
h = Sample size relative to whole plant.
i = Longitudinal sections.
j = Transverse sections.
k = Clear longitudinal sections.
l = Clear transverse sections.
m = Polarized light response.
n = Burn characterization.

**Figure B1.** Data Collection Sheet.



**DATA SHEET** **SPECIMEN NO:** ______________

**METHOD:** ____________________ **RESEARCHER:** Yolanda Olivotto

**DATE:** **START TIME:** **END TIME:**

**METHOD**:
cost:
organizational comments:
(also see lab book entry # ______ . )

**SPECIMEN DESCRIPTION:**
(refer to criteria in method description)
(also see lab book entry, # ______ . )

| SPECIMEN | | | | | PHYTOLITH DETAIL | | |
|---|---|---|---|---|---|---|---|
| DRY MASS gr. | Mineral YIELD gr.; # | Mineral YIELD description | Ash YIELD gr. | Ash YIELD description | DIAGRAM in rotation | SIZE µm;angle | COMMENTS |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

# APPENDIX C:

## Set-up And Per Sample Expenses For Seven Phytolith Distillation Methods

**Table C1.** Set-up and per sample expenses for each method used in the study.

| Method A: Set-up | Canadian $ (1995) | Method A: Per sample | Method B: Set-up | Canadian $ | Method B: Per sample | Method C: Set-up | Canadian $ (1995) | Method C: Per sample |
|---|---|---|---|---|---|---|---|---|
| Microscope (polarizing) | 4000.00 | 0 | Fume Hood | 6000.00 | 0 | Drying Oven | 800.00 | 0 |
| Hardy Microtome | 500.00 | 0 | Beakers and Test Tubes | 60.00 | .10 | Muffle Furnace | 2200.00 | 0 |
| Blade Holder | 160.00 | 0 | Glass Stir Rods (6) | 8.00 | 0 | Crucibles and finger cots | 12.00 | .06 |
| Glass slides (500) | 50.00 | 1.00 | Watch Glass (6) | 9.00 | 0 | Analytical Balance | 2600.00 | 0 |
| Cover Slips (500) | 15.00 | .30 | Wash Bottle | 6.00 | 0 | Burner System including gas | 240.00 | .40 |
| Microscope slide tray (2) | 40.00 | 0 | Chemicals | 180.00 | 2.82 | Desiccater System (6) | 1234.00 | 0 |
| Pipette and Bulb | 20.00 | 0 | Microscope (polarizing) | 4000.00 | 0 | Microscope (polarizing) | 4000.00 | 0 |
| Kimwipes (6 boxes) | 10.00 | .02 | Microscope slide tray (2) | 40.00 | 0 | Microscope slide tray (2) | 40.00 | 0 |
| Razor Blades (50) | 10.00 | .40 | Glass slides & slips (500) | 65.00 | 1.30 | Glass slides & slips (500) | 65.00 | 1.30 |
| Microprobe (2) | 15.00 | 0 | Microscope slide tray (2) | 40.00 | 0 | Microscope slide tray (2) | 40.00 | 0 |
| Microforceps (1) | 40.00 | 0 | Microprobe & Microspatula | 20.00 | 0 | Microprobe (2) | 15.00 | 0 |
| Finger Cots (144 pk) | 5.00 | .06 | Microforceps (1) | 40.00 | 0 | Microforceps (1) | 40.00 | 0 |
| markers (12) (12) | 8.00 | .01 | markers (12) (12) | 8.00 | .01 | markers (12) (12) | 8.00 | .01 |
| deionized water (10L) | 7.50 | .05 | deionized water (20L) | 15.00 | .25 | deionized water (10L) | 7.50 | .05 |
| glycerol | 4.00 | .01 | glycerol & Kimwipes | 14.00 | .03 | glycerol | 4.00 | .01 |
| clear nail polish (2) | 7.00 | .03 | clear nail polish (2) | 7.00 | .03 | clear nail polish (2) | 7.00 | .03 |
| Ethyl Alcohol | 5.00 | .02 | Ethyl Alcohol | 5.00 | .02 | Ethyl Alcohol | 5.00 | .02 |
| **Total** | **4,896.50** | **1.90** | | **10,517.00** | **4.56** | | **11,317.50** | **1.88** |

Table C1. Continued.

| Meth. D-F: Set-up | Canadian $ (1995) | Meth. D-F: Per sample | Method G: Set-up | Canadian $ (1995) | Method G: Per sample |
|---|---|---|---|---|---|
| Muffle Furnace | 2200.00 | 0 | Plasma Ashing Apparatus | 25,000.00 | 0 |
| Furnace Tile | 16.00 | 0 | Oxygen Gas | 75.00 | .05 |
| Glass slides & slips (500) | 65.00 | 1.30 | Glass slides & slips (500) | 65.00 | 1.30 |
| Microscope slide tray (2) | 40.00 | 0 | Microscope slide tray (2) | 40.00 | 0 |
| Microprobe (2) | 15.00 | 0 | Microprobe (2) | 15.00 | 0 |
| Microforceps (1) | 40.00 | 0 | Microforceps (1) | 40.00 | 0 |
| markers (12) (12) | 8.00 | .01 | markers (12) (12) | 8.00 | .01 |
| deionized water (10L) | 7.50 | .05 | deionized water (10L) | 7.50 | .05 |
| glycerol | 4.00 | .01 | Microscope (polarizing) | 4000.00 | 0 |
| clear nail polish (2) | 7.00 | .03 | Finger Cots (144 pk) | 5.00 | .06 |
| Ethyl Alcohol | 5.00 | .02 | Kimwipes (6 boxes) | 10.00 | .02 |
| Microscope (polarizing) | 4000.00 | 0 |  | 0 | 0 |
| Finger Cots (144 pk) | 5.00 | .06 |  | 0 | 0 |
| Kimwipes (6 boxes) | 10.00 | .02 |  | 0 | 0 |
| **Total** | **6,422.50** | **1.50** |  | **29,265.50** | **1.49** |

# APPENDIX D:

## Percent Observed Occurrence Of Phytoliths In The Materials Used In The Study

**Table D1.** Occurrence of phytoliths in *Musa textilis* Née leaf sheath samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 0001a | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 4 |
| | 0001b | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 4 |
| | 0001c | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 4 |
| | 0001d | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 4 |
| | 0001e | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 4 |
| B | 0005a | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| | 0005b | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| | 0005c | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| | 0005d | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| | 0005e | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| C | 0010a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 0011a | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 0012a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 0013a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| Tot.©phytoliths | | 14 | N/A | 5 | 11 | N/A | 14 | 5 | 49 |
| Tot. samples | | 14 | N/A | 14 | 14 | N/A | 14 | 14 | 70 |
| % ©phytoliths | | 100% | N/A | 36% | 79% | N/A | 100% | 36% | **70%** |

**Note.** 1 = yes; 0 = no, or not discernable; N/A = an entire column of 0; 0001c also had "glassy" element with ochre, articulated protrusion.

**Table D2.** Occurrence of phytoliths in *Musa textilis* Née wide strip (WS) grade fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 100a | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 100b | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 100c | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 100d | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| B | 102 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 106 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 107 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| C | 124 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 125 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 126 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 127 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 128 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| D | 108a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 108b | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 108c | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 108d | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| E | 113a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 113b | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 113c | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 113d | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| F | 109a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 109b | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 109c | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 109d | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| G | 116a | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Tot.©phytoliths | | 25 | N/A | N/A | 7 | N/A | 20 | N/A | 52 |
| Tot. samples | | 25 | N/A | N/A | 25 | N/A | 25 | N/A | 75 |
| % ©phytoliths | | 100% | N/A | N/A | 28% | N/A | 80% | N/A | **69%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D3.** Occurrence of phytoliths in *Musa textilis* Née good (G) grade fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhomboid crystal | Calcium oxalate druse crystal | Grand Total Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 230a | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 230b | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 230c | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 230d | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 230e | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| B | 242a | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 242b | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 242c | 1 | 0 | 0 | 1 | X | 0 | 0 | 2 |
| | 242d | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 242e | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| C | 210 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 211 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 212 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 213 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 214 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| E | 200 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 201 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 202 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 203 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 204 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| G | 206 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 206b | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 206c | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 206d | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 206e | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Tot.©phytoliths | | 25 | N/A | N/A | 9 | N/A | 6 | N/A | 40 |
| Tot. samples | | 25 | N/A | N/A | 25 | N/A | 25 | N/A | 75 |
| % ©phytoliths | | 100% | N/A | N/A | 36% | N/A | 24% | N/A | **53%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0;
X = potential cross contamination from Agave.

**Table D4.** Occurrence of phytoliths in *Musa textilis* Née paper samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 250a | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 250b | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 250c | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| | 250d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 250e | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| C | 260 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 261 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 262 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 263 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| E | 265a | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 265b | 0.5 | 0 | 0 | 0 | 0 | 1 | 0 | 1.5 |
| | 265c | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| | 265d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 265e | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Tot.©phytoliths | | 10 | N/A | N/A | N/A | N/A | 4 | N/A | 14 |
| Tot. samples | | 14 | N/A | N/A | N/A | N/A | 14 | N/A | 28 |
| % ©phytoliths | | 71.43% | N/A | N/A | N/A | N/A | 28.57% | N/A | **50%** |

**Note.** 0.5 = fragments; 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0. The low overall percentage of phytoliths that were observed was due to the difficulty of viewing the mechanically disaggregated samples.

**Table D5.** Occurrence of phytoliths in *Musa textilis* Née pulp samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 270a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | 270b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | 270c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 276 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
|  | 267 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
|  | 278 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
|  | 279 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| Tot.©phytoliths |  | 4 | N/A | N/A | N/A | N/A | 4 | N/A | 8 |
| Tot. samples |  | 7 | N/A | N/A | N/A | N/A | 7 | N/A | 14 |
| % ©phytoliths |  | 57.14% | N/A | N/A | N/A | N/A | 57.14% | N/A | **57%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D6.** Occurrence of phytoliths in *Musa textilis* Née "linen" samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 300a | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 300b | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 300c | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 300d | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 300e | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| C | 310 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 311 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 312 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 313 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| E | 320a | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 320b | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 320c | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 320d | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Tot.©phytoliths | | 13 | N/A | N/A | N/A | N/A | 4 | N/A | 17 |
| Tot. samples | | 13 | N/A | N/A | N/A | N/A | 13 | N/A | 26 |
| % ©phytoliths | | 100% | N/A | N/A | N/A | N/A | 30.77% | N/A | **65%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D7.** Occurrence of phytoliths in *Musa*, species not *textilis*, residual grade fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 1100a | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1100b | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1100c | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1100d | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1100e | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| B | 1101a | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1101b | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1101c | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| | 1101d | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| E | 1103a | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 1103b | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 1103c | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | 1103d | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | 1103e | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 3 |
| Tot.©phytoliths | | 14 | N/A | N/A | 12 | N/A | 11 | N/A | 37 |
| Tot. samples | | 14 | N/A | N/A | 14 | N/A | 14 | N/A | 42 |
| % ©phytoliths | | 100% | N/A | N/A | 85.71% | N/A | 78.57% | N/A | **88%** |

**Note**. 1 = yes; 0 = no, or not discernable; N/A = an entire column of 0.

**Table D8.** Occurrence of phytoliths in *Agave cantala* leaf samples, and *Agave* course fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| Agave leaf A | 350 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | | | | | | | | | |
| Agave fibre A | 400a | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 400b | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 400c | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 400d | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 400e | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Agave fibre B | 410a | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 410b | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 410c | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 410d | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| | 410e | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| Agave fibre E | 420a | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 420b | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 420c | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 420d | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 420e | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| G | 426a | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tot.©phytoliths | | N/A | N/A | N/A | 8 | 16 | N/A | N/A | 24 |
| Tot. samples | | N/A | N/A | N/A | 16 | 16 | N/A | N/A | 32 |
| % ©phytoliths | | N/A | N/A | N/A | 50% | 100% | N/A | N/A | **75%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D9.** Occurrence of phytoliths in *Agave* rope samples treated with various methods

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | GrandTotal: Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 450a | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 450b | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 450c | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 450d | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 450e | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| E | 465a | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 465b | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 465c | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 465d | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 465e | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tot.©phytoliths | | N/A | N/A | N/A | N/A | 10 | N/A | N/A | 10 |
| Tot. samples | | N/A | N/A | N/A | N/A | 10 | N/A | N/A | 10 |
| % ©phytoliths | | N/A | N/A | N/A | N/A | 100% | N/A | N/A | **100%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D10.** Occurrence of phytoliths in *Boehmeria nivea* fibre ultimate samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 500a | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 500b | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 1 |
| | 500c | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0.5 |
| | 500d | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0.5 |
| | 500e | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| B | 510a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 510b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 510c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 510d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 510e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E | 520a | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 520b | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 520c | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 520d | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 520e | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| G | 526a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 526b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tot.©phytoliths | | N/A | N/A | 8.5 | N/A | N/A | 0.5 | 6 | 15 |
| Tot. samples | | N/A | N/A | 17 | N/A | N/A | 17 | 17 | 51 |
| % ©phytoliths | | N/A | N/A | 50% | N/A | N/A | 3% | 35% | **29%** |

**Note.** 0.5 = fragments; 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D11.** Occurrence of phytoliths in *Corchorus capsularis* fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | GrandTotal: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| A | 600a | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 600b | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 600c | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 600d | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 600e | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| B | 610a | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 610b | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 610c | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 610d | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 610e | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| E | 620a | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 620b | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 620c | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 620d | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 620e | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| G | 626a | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
|  | 626b | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Tot.©phytoliths |  | N/A | N/A | N/A | N/A | N/A | 17 | N/A | 17 |
| Tot. samples |  | N/A | N/A | N/A | N/A | N/A | 17 | N/A | 17 |
| % ©phytoliths |  | N/A | N/A | N/A | N/A | N/A | 100% | N/A | **100%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D12.** Occurrence of phytoliths in *Cannabis sativa* stem samples and *summer cloth* samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total: Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| Stems A | 700a | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| | 700b | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| | 700c | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| | 700d | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| | 700e | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| | | N/A | 5 | 5 | N/A | N/A | 2 | 5 | 15 |
| Cloth A | 800a | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| | 800b | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| | 800c | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | 800d | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| | 800e | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| B | 810a | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| | 811 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| | 812 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| | 814 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| E | 820a | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | 820b | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | 820c | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | 820d | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | 820e | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| G | 826a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 826b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tot.©phytoliths | | N/A | 6 | 4 | N/A | N/A | N/A | 13 | 22 |
| Tot. samples | | N/A | 16 | 16 | N/A | N/A | N/A | 16 | 48 |
| % ©phytoliths | | N/A | 37.5% | 25% | N/A | N/A | N/A | 81.3% | **46%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D13.** Occurrence of phytoliths in *Agave sisalana* rope fibre samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total Diff. forms |
|---|---|---|---|---|---|---|---|---|---|
| E | 1270a | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 1270b | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 1270c | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 1270d | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 1270e | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tot.©phytoliths | | N/A | N/A | N/A | N/A | 5 | N/A | N/A | 5 |
| Tot. samples | | N/A | N/A | N/A | N/A | 5 | N/A | N/A | 5 |
| % ©phytoliths | | N/A | N/A | N/A | N/A | 100% | N/A | N/A | **100%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

**Table D14.** Occurrence of phytoliths in *Spinacia oleracea* leaf samples treated with various methods.

| Method | Sample number | Silica body, troughed | Silica body, trichome base | Calcium oxalate crystal sand | Calcium oxalate raphide crystal | Calcium oxalate styloid crystal | Rhom-boid crystal | Calcium oxalate druse crystal | Grand Total Diff forms |
|---|---|---|---|---|---|---|---|---|---|
| B | 1310 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| G | 1326 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Tot.©phytoliths | | N/A | N/A | N/A | N/A | N/A | N/A | 2 | 2 |
| Tot. samples | | N/A | N/A | N/A | N/A | N/A | N/A | 2 | 2 |
| % ©phytoliths | | N/A | N/A | N/A | N/A | N/A | N/A | 100% | **100%** |

**Note.** 1 = yes; 0 = no, or not discernible; N/A = an entire column of 0.

# APPENDIX E:

## Additional collected data which is referenced, but not discussed in the study.

**Table E1.** Burn characteristics and twist test results from the materials researched.

| *Material* | Twist Test Results | Burn Charateristics | | | | | |
|---|---|---|---|---|---|---|---|
| | | near flame | at ignition | in flame | flame colour | out of flame | ash colour & texture |
| *Musa textilis*, leaf sheath | clockwise | does not smoke; draws flame towards itself | ignites 0.5 mm from flame | combusts but only to curled, black charred remains | no affect | extinguish in <10 sec.; no ash drops away | brittle and granular black ash |
| *Musa textilis*, WS grade fibre | " | ignites 2 mm from flame | " | burns red and curls clockwise | " | extinguish slowly; no ash drops away | ashed white throughgray, to black, & brittle |
| *Musa textilis*, G grade fibre, and linen | " | ignites 4 mm from flame | burns quickly | " | " | " | " |
| *Musa textilis*, paper product, and pulp product | " | " | " | burns and curls clockwise | " | no ash drops away | ash is white mat of brittle "fibres", 0.5 µm; grainy, greasy texture |
| *Agave cantala*, leaf | clockwise | no ignition | ignites in flame | sweet *Cannabis* smoke | orange at tip | extinguish immediate, retains form; no ash drops | white & brittle; powders between finger tips |
| *Agave*, coarse fibre | " | ignites 2 mm from flame | not self-sustain | burns slowly and complete | " | " | " |
| *Agave*, rope fibre | " | " | self-sustain | | " | " | " |
| *Boehmeria nivea*, approx. 20 ultimates | counter-clockwise | ignites 3-4 mm from flame | curls away & extinguishes | fast "pffft" and fibre ashing complete | no affect | no ash drops, tightly curled. | cream fibrous ash; crush to gritty powder |
| *Boehmeria nivea*, approx.800 ultimates | " | " | self-sustain | curls counter-clockwise as burns | " | " | " |
| *Corchorus capsularis* | clockwise | ignites 2 mm from flame | fine fibres curl & extinguish | burns quickly and curls | no affect | no ash drops, some curling. | Gray to black powder, no gritty texture |
| *Cannabis sativa*, stem | clockwise | ignites 1 mm from flame | pith smokes | burns slowly with "classic" *Cannabis* aroma | flame orange; black smoke | self sustained burn; no ash drops away | black with white dot at end; black ash is granular |

PLATE E1.

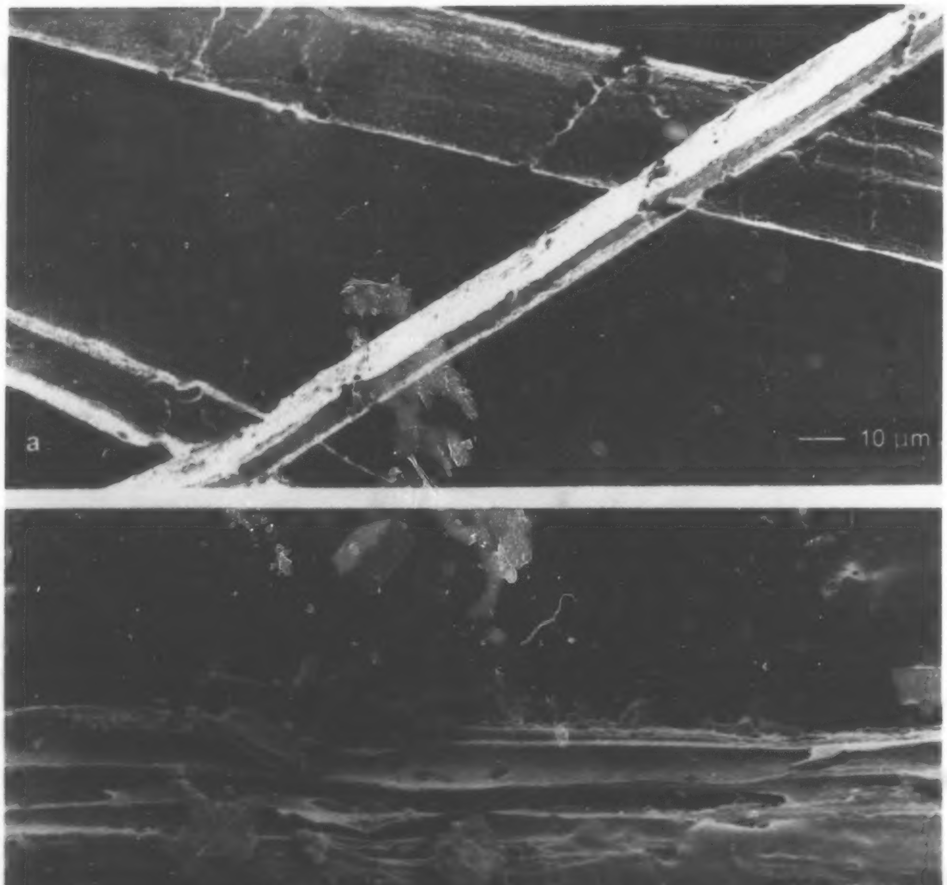
## PLATE E1.

**Three scanning electron micrographs of oxygen plasma ashed fibres.**

**a)** *Boehmeria nivea* (L.) Gaud. **(ramie).** The nodes, amorphous regions, have been degraded in a diagonal pattern, but the fibres are smooth relative to *Cannabis*.

**b)** *Agave*, species not determined, may be *cantala* (**maguay**), or *fourcroydes* (**henequen**). The fibre cell walls are degraded in an overall way, exposing the cell lumen.

**c)** *Cannabis sativa* L. (**hemp**). The node regions are degraded, and the fibre cell walls are unevenly digested.


a
10 µm
b
100 µm
c
10 µm